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COMPARISON OF DIPLOID AND TRIPLOID SUGAR BEETS[1]

BY F. H. PETO[2] AND J. W. BOYES[2]

Abstract

In a small replicated field trial, percentage sugar and root weight were negatively correlated in both diploid and triploid individual beets, but the decreases in percentage sugar for every 100-gm. increase in root weight differed significantly, being on the average 0.34% for the diploids and 0.17% for the triploids. As root weight increased beyond 900 gm. the yield of sugar per 100 gm. of root weight increased more rapidly in the triploids than in the diploids. Under the conditions of this test, triploid beets exceeded the diploids in root weight by 12.2%, in yield of sugar per beet by 14.9%, and in dry top weight by 17.8%. The triploids exceeded the diploids in area index of the leaves by 34.4% and in area index of the stomata by 42.6%.

Induced Tetraploidy in Sugar Beets and the Origin of Triploid Progeny

Colchicine treatments of diploid sugar beet stecklings from a selection of the variety "Great Western" resulted in the production of tetraploid sectors or branches. The colchicine was dissolved in an agar gel and applied in gelatine drug capsules which had been previously waterproofed with a coating of collodion. The young shoots or branch tips were decapitated and capsules containing 0.1 or 0.5% colchicine were slipped over the cut end. The colchicine affected the axillary buds just below the point of decapitation, and by cutting back all normal shoots, growth in the affected shoots was promoted. Abnormally large pollen grains were found on certain branches of five of the 33 stecklings treated. Chromosome counts were made on 206 of the progeny of affected branches, and 69 triploids and two tetraploids were found. It was obvious that the majority of the tetraploid flowers must have been fertilized with pollen from diploid sectors of the same plant or from adjacent diploid plants.

At approximately the same time that the above triploids and tetraploids were produced, Frandsen (2), Rasmusson and Levan (10), and Schwanitz (13) were also successful in obtaining tetraploids by colchicine treatments, but the method of application differed in each case from that outlined above.

[1] *Manuscript received March 4, 1940.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. N.R.C. No. 920.

[2] *Cytogeneticist.*

Methods of Testing Diploid and Triploid Beets

Fifty-six triploids and the same number of diploids were obtained from seed sown in the greenhouse on January 11, 1939. They were transplanted to the field on May 29 and planted in rows of seven plants with diploid and triploid rows alternated. The rows were spaced 22 in. apart and the beets planted 10 in. apart in the rows. At the time of planting, the triploids were slightly larger than the diploids, and the difference in size became more pronounced as the season advanced. Unfortunately, very heavy rains in late July and mid-August caused temporary flooding over three-quarters of the experimental plots. This checked development greatly in the area affected and resulted in a very wide range in beet size. The plots were replicated in such a manner that the diploids and triploids appeared to be equally affected so that the experiment was not lost, although the conditions throughout development were by no means typical of normal commercial conditions where seed is sown directly in the field in the spring and the plants are grown under normal moisture conditions. Nevertheless, information on the relative behaviour of diploid and triploid beets under these conditions should be of value.

Leaf dimensions of five of the largest leaves on each plant were taken on August 8. A slight difficulty was encountered in determining the length of the lamina owing to differences in the form of the leaf base. However, one observer took all the measurements, and maximum length and width of the lamina were recorded.

Epidermal strips were taken from the lower surfaces of two well developed leaves of each diploid and triploid plant on August 9. These were fixed in absolute alcohol 3 parts, and acetic acid 1 part. They were later examined, and the length and total width of ten pairs of guard cells from each plant were measured.

The beets were harvested on October 4. The tops were removed just below the lowest living leaf scar, weighed immediately, and the dry weight was obtained after passing them through a forage crop drier. The roots were washed, dried, and weighed in preparation for sugar analysis.

Individual beets were analysed for sugar by hot water digestion, Method I (1). Ten cc. of lead acetate, 0.25 cc. of concentrated acetic acid, and two drops of ether were the quantities of reagents used per determination. The percentage sugar was determined by polarimetric readings obtained with a Hilger polarimeter with 200 mm. tubes and a sodium lamp. A factor of 0.7519 was used for conversion to percentage sugar and 200/26 for dilution correction, the combined factor being 5.7838.

Preliminary tests revealed the difficulty of obtaining a representative sample from the beet. This was finally overcome by dividing the root longitudinally and grinding one half in a meat chopper, using a plate with $\frac{1}{4}$-in. perforations. Before taking the 26-gm. sample, the pulp was mixed thoroughly with a spoon for approximately 90 sec. In this way it was possible

to obtain agreement between duplicate samples to within 0.1% sugar on the average, with a maximum difference of 0.2%. Duplicate samples were taken from every fifth beet and single determinations were run on the remainder.

Percentage of Sugar and Root Weight

Several investigators have studied the relation between percentage sugar and root weight. Pack (8) summarized the evidence prior to 1930 and concluded that there is, in general, a negative correlation between these two characters. Subsequent studies by Immer (4) and Garner and Sanders (3) support this general conclusion. A summary of the data by several authors as shown in Table I reveals that the correlation coefficients secured vary from about −0.2 to −0.7 and that the average percentage decrease in sugar per increase of 100 gm. in root weight varies from 0.10 to 0.39. In one instance a significant positive correlation was reported.

TABLE I

COMPARISON OF THE RESULTS OF VARIOUS AUTHORS WITH REGARD TO THE CORRELATION OF PERCENTAGE SUGAR AND ROOT WEIGHT

Author	Remarks	Average % change in sugar for increase of 100 gm. in root weight	Correlation coefficients
Rümker (11)		−0.29	
Novotný (6)		−0.39 to −0.19	
Pack (8)			−0.53 ± 0.05 −0.52 ± 0.05 −0.48 ± 0.06 −0.33 ± 0.06 −0.27 ± 0.06
Immer (4)		−0.13	−0.26
Oetken (7)		−0.10	
Garner and Sanders (3)	Spacing 12 × 6 in. Spacing 18 × 9 in.	+0.51 −0.19	+0.45* −0.34*
Peto and Boyes	Diploids Triploids	−0.34 −0.17	−0.74** −0.54**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Genetical differences in the strains tested may account for some of the variation noted in Table I. For example, Pack (8) studied five very different lines and found that r values ranged from −0.27 to −0.53. Under strictly comparable environmental conditions, Garner and Sanders (3) studied the effect of various spacings and revealed that environmental conditions may influence the relation between sugar and root weight. Close spacings of

12 × 6 in. during a hot dry season resulted in a correlation coefficient of +0.451 and 0.51% increase in sugar percentage for 100 gm. increase in root weight. Wider spacings of 18 × 9 in. during a more favourable season gave a correlation coefficient of −0.336 and 0.19% decrease in sugar percentage for 100 gm. increase in root weight. The authors point out that the beets giving the positive correlations were very small, averaging only 163 gm. each, which shows the extremely unfavourable environmental conditions under which they developed.

In the present investigation the correlation coefficient between percentage of sugar and weight of individual beets for the diploids is −0.74 and for the triploids is −0.54. The r value for the diploids is much higher than any other values listed in Table I, whereas the triploid r value is similar to the higher values reported by Pack (8) and Garner and Sanders (3).

TABLE II

TESTS OF SIGNIFICANCE OF DIFFERENCES IN DIPLOID AND TRIPLOID REGRESSION FUNCTIONS

Regression of	Source of variance	D.f.	Mean square
Yield of sugar on root weight	Difference in regression coefficients	2	1,976**
	Residuals	106	135
Percentage sugar on root weight	Difference in regression coefficients	1	12.47**
	Residuals	108	1.30
Yield of sugar on dry top weight	Difference in regression coefficients	2	1,621
	Difference in centroids	1	717
	Residuals	106	752
Root weight on dry top weight	Difference in regression coefficients	1	1
	Difference in centroids	1	33
	Residuals	108	38,492

** *Exceeds 1% level of significance.*

The diploids and triploids differed significantly in regard to slope of their regression lines, the differences exceeding the 1% level of significance (Table II). The average decrease in percentage sugar for every 100 gm. increase in root weight was 0.34% for the diploids and 0.17% for the triploids. The data are illustrated in Fig. 1, in which the more nearly horizontal triploid curve shows quite clearly that percentage sugar does not decrease with increased size of root as rapidly in triploid as in diploid beets. This difference cannot justifiably be ascribed to environmental differences nor to any great extent to qualitative differences in genic constitution, since the triploid originated from the diploid material. Consequently the addition of an extra set of chromosomes must have affected the slope of the line of regression of percentage sugar on root weight.

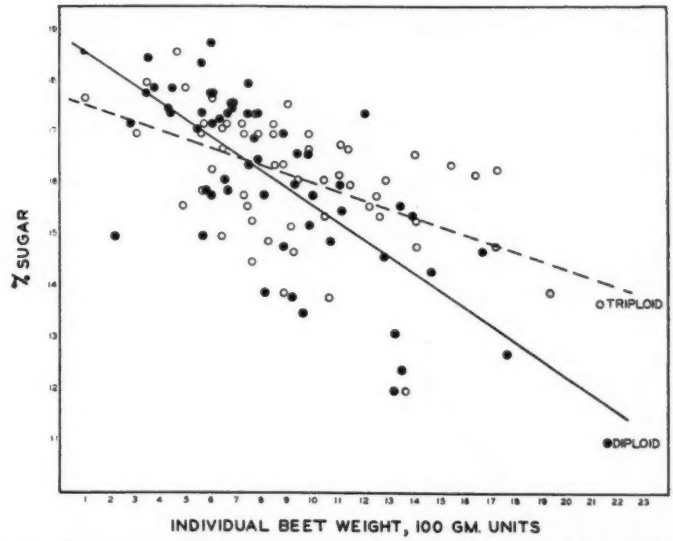

FIG. 1. *Relation between percentage sugar and root weight in individual diploid and triploid beets.*

Yield of Sugar and Root Weight

The most valuable single agronomic characteristic of sugar beets is yield of sugar. Consequently its relation to weight of root is of considerable importance. The previous section, dealing with the regression of percentage of sugar on root weight, showed that the regressions of diploids and triploids had different slopes, though it is possible that the relation is not truly linear throughout the complete range. However, the extent of scatter and low correlation of the two characters limited the interpretation to a linear expression. The determination of the regression of yield of sugar on root weight overcame this difficulty, since these two characters were closely correlated, the r values being $+0.97$ for diploids and $+0.98$ for triploids. The shape of the respective diploid and triploid curves (Fig. 2) was determined by the percentage of sugar, since root weight was a function in both characters under consideration. The diploid and triploid regression lines differed strikingly in degree of curvature beyond the midpoint, at which the diploid curve began to fall away rapidly from the more nearly linear triploid curve. The significance of this difference exceeded the 1% point (Table II) in spite of the fact that the curves were almost identical for beets smaller than 900 gm. It seems possible that the adverse growth conditions resulting from temporary flooding in the area producing the small beets suppressed growth to an extent that tended to mask the difference in the diploids and triploids within this size range.

The difference in the slope of the diploid and triploid regression lines for the larger beets indicates a fundamental difference which may prove to be

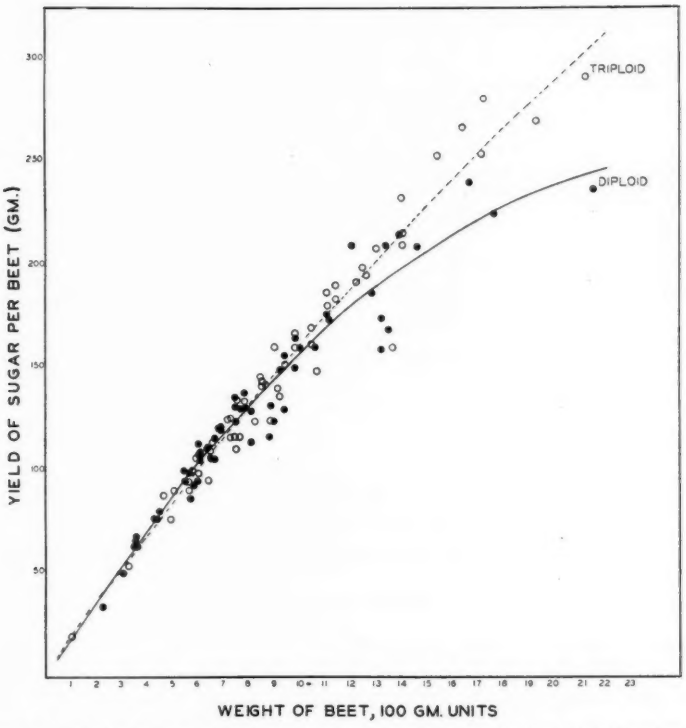

FIG. 2. *Relation between yield of sugar and root weight in individual diploid and triploid beets.*

of considerable practical importance. On the basis of this experiment it should be possible to produce very large triploid beets without any serious reduction in percentage sugar, whereas the economic limit in size might soon be reached in the production of larger diploid beets, where any marked reduction in percentage sugar would counterbalance the advantage of increased root weight.

The Relation of Root Weight and Yield of Sugar to Dry Top Weight

Root weight, as would be expected, was closely correlated with dry top weight, the r values for diploids and triploids being $+0.86$ and $+0.89$ respectively. The diploid and triploid regression lines for these characters were not significantly different. Similar results were obtained when yield of sugar was correlated with dry top weight, the r values for diploids and triploids being $+0.83$ and $+0.87$ respectively. The diploid and triploid regression lines for these characters were also very similar.

Comparison of Diploids and Triploids on a Plot Basis

The "t" test (Snedecor 14) was applied to the means of the eight diploid and triploid plots each containing seven plants. The characters studied were root weight, percentage sugar, yield of sugar, and dry top weight. The results are summarized in Table II. Diploid and triploid single row plots were alternated in the field instead of being randomized as required for proper use of the "t" test. In spite of this defect in plot arrangement, the "t" test was considered to be the most useful test since there did not appear to be any consistent positional relation favouring either the diploid or triploid in this closely replicated series. Nevertheless, undue emphasis must not be placed on the significance of the differences derived in this manner.

The plot means are recorded in Table III to show the adjacent plots that were compared. The triploid plot means exceeded their adjacent diploid plot means in root weight, yield of sugar, and dry top weight with one exception in each case, which was accounted for by the presence of one abnormally small triploid beet.

The mean increase of triploids over diploids for root weight was 12.2%, for yield of sugar 14.9% and for dry top weight 17.8%. These increases were significant according to the "t" value shown in Table III, although the

TABLE III

PLOT MEANS OF DIPLOID AND TRIPLOID SUGAR BEETS

Plot No.	Characters									
	Mean root wt., gm.		Per cent sugar		Yield of sugar		Dry top wt., gm.		Per cent moisture in tops	
	2n	3n	2n	3n	2n	3n	2n	3n	2n	3n
1	1317	1361	14.8	15.0	195	204	153	161	85	85
2	943	964	14.7	15.3	126	145	105	127	83	86
3	580	797	16.2	16.3	92	129	58	75	82	84
4	992	1064	15.5	15.4	147	162	102	125	84	84
5	705	973	16.5	16.1	112	157	54	79	82	83
6	871	991	16.5	16.4	143	158	102	105	84	83
7	585	706	17.6	17.4	102	122	48	78	80	82
8	846	817	16.9	16.9	138	137	104	104	83	83
Mean	854.9	959.0	16.1	16.1	131.9	151.6	90.7	106.8	82.6	83.5
Mean increase of triploids over diploids	104.1		0.0		19.7		16.1		0.9	
Per cent increase	12.2		0.0		14.9		17.8		1.1	
"t" value	2.96*		—		3.80**		4.21**		1.89	

* *Exceeds 5% level (2.36) of significance.*
** *Exceeds 1% level (3.50) of significance.*

percentage increase would likely be very different under more favourable and uniform conditions of growth. The transplanting of well developed seedlings and the unusually long period from germination to harvest may also have affected differentially the diploids and triploids. Consequently additional tests must be made under more normal conditions before the relative qualities of the triploids and diploids could be accurately predicted.

Although the triploids had larger roots on the average than the diploids, the mean percentage of sugar was the same for both. It can be concluded from this that the triploids on the average must have contained a higher percentage of sugar for a given size, as was illustrated by the regression curves.

The diploids and triploids did not differ significantly in regard to percentage moisture in the tops.

Leaf and Stomatal Dimensions

The length and width of the lamina of five of the largest leaves of each plant were taken, and the plot means of these dimensions together with the length-width ratio and area index (length × width) are recorded in Table IV. The triploids exceeded the diploids in length of leaf (lamina) by 15.6%, in width of leaf by 16.8% and in area index by 34.4%. The ratio of length to width was very similar for diploids and triploids, which is in contrast to the observation by Rasmusson and Levan (10) that the triploids have shorter and broader laminae than the diploids.

TABLE IV

PLOT MEANS FOR LEAF DIMENSIONS (INCHES)

Plot No.	Leaf length (in.)		Leaf width (in.)		Ratio L/W		Area index ($L \times W$)	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
1	8.6	10.8	5.9	6.6	1.54	1.64	50.97	71.11
2	9.1	10.2	5.4	6.3	1.69	1.63	49.50	63.66
3	7.1	9.0	4.8	6.0	1.48	1.50	33.68	54.24
4	9.7	10.4	5.6	6.6	1.72	1.58	54.82	68.73
5	7.9	9.4	4.9	5.9	1.62	1.58	38.83	55.50
6	9.3	10.5	5.8	6.2	1.60	1.68	53.58	65.33
7	7.1	8.6	4.3	5.7	1.64	1.50	30.54	49.34
8	8.3	8.7	5.2	5.6	1.59	1.56	42.87	48.98
	8.39	9.70	5.24	6.12	1.601	1.584	44.349	59.612
Per cent increase		15.6		16.8	1.1			34.4

The plot means for stomatal dimensions are given in Table V. The triploid stomata exceed the diploid in length of the guard cells by 34.6% and in width by 9.3%. The ratio of length to width in the diploids is 1.05 and in the triploids is 1.20, indicating quite a marked difference in shape. However, stomatal shape would vary so greatly with cell turgor that this difference may

TABLE V

PLOT MEANS FOR STOMATAL DIMENSIONS

(1 unit = 1.3 μ)

Plot No.	Length		Width		Area index $L \times W$	
	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
1	23.6	28.5	20.0	22.3	472.0	635.5
2	21.1	26.9	20.0	21.9	422.0	589.1
3	20.6	26.3	19.0	22.9	391.4	579.4
4	19.4	25.7	18.1	20.9	351.1	537.1
5	22.0	25.7	18.6	22.5	409.2	578.2
6	21.0	27.0	18.3	22.0	384.3	594.0
7	19.7	24.9	18.1	21.5	356.6	535.3
8	20.6	24.4	19.7	20.6	405.8	502.6
Mean	21.00	26.17	19.97	21.82	399.0	568.9
Per cent increase		34.6		9.3		42.6

be of little significance. The stomatal area index of the triploids exceeds the diploids by 42.6%, which parallels the increase of 34.4% in area index of the leaves. The biological significance of this relation cannot be determined at present on the limited information available, but it suggests that the number of cell divisions is similar in diploids and triploids, and that increase in organ size tends to be in direct proportion to increase in cell size.

Discussion

The recent development of artificial methods of inducing chromosome doubling has opened up new possibilities in plant improvement. Chromosome doubling frequently results in increased size of all the plant organs and in addition frequently affects the chemical constitution of plants. Sullivan and Myers (15) found that tetraploid *Lolium perenne* plants contained more reducing sugar and sucrose than the diploid material. Sansome and Zilva (12) found that tetraploid strains of tomato exceeded diploid strains in the vitamin C content. Randolf and Hand (9) report that tetraploid strains of yellow corn were higher in carotinoids than the diploids. Kostoff and Axamitnaja (5) found that tetraploid tomatoes contained a higher proportion of nitrogen and protein than the diploids.

In certain induced autotetraploids the advantage is size, and chemical constitution is frequently nullified by a reduction of seed yield especially in those crops where seed is the merchantable product. However, in the sugar beet the cost of seed is so small in proportion to the cost of production of the roots that a considerable increase in seed cost could be readily borne by any increase in value of the crop through improvement in root yield or quality.

The relative value of tetraploid and diploid sugar beets has not yet been established, but the present investigation indicates that triploids may be

superior to diploids in respect to yield of sugar, weight of root, and particularly in the maintenance of percentage sugar with increased size of root. If further tests substantiate these findings, then the development of methods of producing triploid seed on a commercial scale will require considerable attention. Triploids obviously will not breed true, consequently it will be necessary to hybridize tetraploid and diploid strains to provide the triploid seed.

The production of a very high proportion of triploid seeds from open fertilization of the tetraploid flowers in this experiment indicates the ease with which cross fertilization of tetraploids and diploids may be accomplished. This could likely be repeated in large scale plantings by interspacing tetraploids and diploid stecklings in such a manner that most of the seed produced on the tetraploid was the result of pollination by pollen from diploid plants. There is also the possibility that pollen from diploids is more viable on tetraploid stigmas than pollen from tetraploid plants. Such a condition would greatly facilitate the production of triploid seed. A further advantage in obtaining a high degree of cross fertilization would undoubtedly result from heterosis, and parental tetraploid and diploid strains could be selected with the object of obtaining maximum hybrid vigour. The resulting triploid progeny should combine the advantages inherent in triploidy and heterosis.

Acknowledgments

The authors are indebted to Dr. T. M. Stevenson and Mr. F. Dimmock of the Division of Forage Plants, Central Experimental Farm, Ottawa, for growing the plants, for determining the dry weight of the tops, and for helpful suggestions in the conduct of the experiment; to Dr. H. D. Brown of the Canada and Dominion Sugar Company, Chatham, Ontario, for supplying the original diploid stecklings; and to Mr. M. W. Thistle of the staff of the Division of Biology and Agriculture, who made the statistical computations.

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EFFECTS OF DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE ON THE ROOTING OF DORMANT *LONICERA TARTARICA* CUTTINGS[1]

By N. H. Grace[2]

Abstract

Indolylbutyric acid and oestrone, separately and in combination in a series of talc dusts, were applied to dormant cuttings of *Lonicera tartarica*. Indolylbutyric acid treatment, averaged over all oestrone treatments, increased the number of cuttings rooted, the number and length of roots per rooted cutting, the mean root length, and the green weight of leaf produced by the dormant cuttings. The average effect of oestrone on these responses was depressive throughout. Oestrone tended to offset the beneficial effects of the plant growth stimulating chemical, excepting the combination of 100 p.p.m. of each which increased root length. Oestrone alone did not reduce either the number or lengths of root per rooted cutting.

The results indicated that the number and length of roots and the green weight of leaf produced are more sensitive responses for demonstrating differences due to indolylbutyric acid concentration in talc than is the percentage of cuttings rooted.

An earlier communication has described some effects obtained with dormant cuttings on treatment with indolylbutyric acid and oestrone (2). While the dust treatments with oestrone had no significant effects on the various responses, colloidal solutions of this chemical, both alone and in combination with indolylbutyric acid, affected the weight of green leaf produced by the dormant cuttings. In view of the effects on top growth and the indication of some interaction between these plant and animal hormones, it was considered of interest to carry out an experiment in which both these chemicals were applied, separately and in conjunction with each other, by means of a dust carrier. The present communication describes the results of such an experiment.

Experimental

The effects of the two chemicals, indolylbutyric acid and oestrone, were investigated by an experiment of factorial design. The experiment involved a series of 16 dust mixtures comprising indolylbutyric acid at four concentrations, namely 0, 100, 500, and 1000 p.p.m. (parts of chemical per million parts of talc by weight) in combination with oestrone at the same four concentrations. The individual concentrations and the various mixtures were readily made from a 2000 p.p.m. preparation of each chemical by admixture and addition of ball-milled talc. The 2000 p.p.m. dusts had been prepared by the grinding mix method, a procedure that ensured thorough distribution of the chemical in the carrier.

[1] *Manuscript received March 23, 1940.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as N.R.C. No. 919.

[2] *Biochemist.*

Dormant cuttings‡ of *Lonicera tartarica* L. were received on November 2, 1939, and treated in groups of 10 cuttings with the 16 different dust preparations. Prior to dusting the cuttings were sprayed with water (3). There were four replicates of the 16 treatments with complete randomization of order of planting within each replicate; the entire experiment involved the use of 640 cuttings. The dusted cuttings were planted immediately in brown sand in a propagation bed provided with electrical bottom heat cables. The room temperature approximated 65° F. and that of the sand about 70° F. The cuttings were removed for observation on December 18, or 46 days after planting.

A record was made of the number of cuttings rooted in each group of 10, the number and length of the primary roots, and the weight of green leaf per group of cuttings. The mean root length was calculated from the number and total lengths of roots. All the data obtained were subjected to the analysis of variance procedure. The numbers of cuttings rooted were subjected to the inverse sine transformation for statistical analysis (1).

Results

The analyses of variance of the responses are given in Table I. In each case the average effect of indolylbutyric acid treatment attained the 0.1% level of statistical significance. On the average, oestrone treatments were

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF DORMANT *Lonicera tartarica* CUTTINGS TREATED WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Source of variance	D.f.	Mean square				
		Number of cuttings rooted	Number of roots per rooted cutting	Length of root per rooted cutting	Mean root length	Weight of green leaf produced per group of 10 cuttings
Between replicates	3	206.1	7.55**	17,443*	1622.5***	0.3006
Treatments with						
Indolylbutyric acid	3	1376.6***	14.97***	185,422***	3120.8***	5.0989***
Oestrone	3	1244.6***	24.06***	81,637***	407.5†	4.4152**
Interaction						
Indolylbutyric acid × oestrone	9	202.6	7.76***	18,827**	157.1	0.9718
Error	45	111.1	1.71	4,558	157.2	0.6130

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
† *Partition of the sum of squares indicated that treatment with 1000 p.p.m. oestrone differed from the mean of the other three levels taken together.*

‡ The prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.

also highly significant with measurements on the mean root length an exception. Partition of the sum of squares for the three degrees of freedom for the effects of oestrone treatment on the mean root length indicated that the 1000 p.p.m. oestrone treatment differed from the average value for the other three levels taken together; the significance attained was the 5% level. Significant interaction between chemicals was shown by the observations on numbers and lengths of root per rooted cutting; there was a suggestion of an interaction shown by counts on the number of cuttings rooted but the mean square fell short of the 5% level of significance, a condition not changed by partition of the sum of squares for the interaction.

TABLE II

AVERAGE RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS TREATED WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Data are means for 16 groups of 10 cuttings

	Indolylbutyric concentrations, p.p.m. (average over all oestrone concentrations)				Oestrone concentrations, p.p.m. (average over all indolylbutyric concentrations)				Necessary difference, 5% level
	0	100	500	1000	0	100	500	1000	
Number of cuttings rooted									
Transformed data	43.5	60.4	64.9	59.0	69.3	56.1	53.8	48.6	7.5
Per cent	47.5	70.6	78.1	70.0	80.6	66.9	63.1	55.6	
Number of roots per cutting rooted	3.9	4.7	6.1	5.8	6.8	5.1	4.4	4.1	0.93
Length of root per rooted cutting, mm.	67	138	285	279	284	211	154	121	48
Mean root length, mm.	21.5	28.8	47.8	49.8	37.6	41.6	38.9	29.8†	8.9
Weight of green leaves per group of 10 cuttings, gm.	1.78	2.78	3.08	2.34	3.01	2.84	2.09	2.02	0.55

† *See footnote, Table 1. The 1000 p.p.m. concentration of oestrone gave a mean root length of 29.8 mm., the mean of the other oestrone concentrations, 39.4 mm.; both values averaged over all indolylbutyric acid treatments and the only significant effect of oestrone treatment on the mean root length.*

In Table II are given data for the average responses of indolylbutyric acid and oestrone treatments, the result for each chemical being averaged over the four concentrations of the other. All indolylbutyric acid treatments increased the number of rooted cuttings and it is of interest to point out that there was no significant difference in rooting over the range from 100 to 1000 p.p.m., though an optimum was suggested at the 500 p.p.m. level. Oestrone treatment reduced rooting, the 100 and 500 p.p.m. concentrations did not differ between themselves, but the 1000 p.p.m. was below the 100 p.p.m. concentration, suggesting increase in injury to rooting with ascending concentration. Indolylbutyric acid treatment at the 100 p.p.m. concentration suggested an increase in mean root length; the increase became highly signi-

ficant at the two higher concentrations, which failed to differ between themselves. The 1000 p.p.m. oestrone treatment gave a mean root length of 29.8 mm., a value significantly below 39.4 mm., the mean value for the 0, 100, and 500 p.p.m. concentrations taken together. The weight of green leaves per group of 10 cuttings was greater for all treatments with indolylbutyric acid than for the controls; the 500 p.p.m. concentration was followed by the greatest weight of new growth; a significant reduction in the leaf weight over that at the 500 p.p.m. concentration followed the 1000 p.p.m. concentration. While the decrease in leaf weight on treatment with 100 p.p.m. oestrone was not significant, the decrease was marked at the two higher levels, which failed to differ between themselves.

TABLE III

NUMBER OF ROOTS PER ROOTED DORMANT *Lonicera tartarica* CUTTING ON TREATMENT WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Data are means from 4 groups of 10 cuttings

Oestrone treatments, p.p.m.	Indolylbutyric acid treatments, p.p.m.			
	0	100	500	1000
0	3.9	4.9	9.9	8.7
100	4.2	5.3	5.0	6.1
500	4.3	4.5	5.0	3.7
1000	3.4	4.3	4.3	4.6

Necessary difference, 5% level, 1.86 roots.

Data are given in Table III for the interaction effects of indolylbutyric acid and oestrone on the number of roots per rooted cutting. The interaction between chemicals is such that in the absence of oestrone, both the 500 and 1000 p.p.m. treatments of indolylbutyric acid resulted in a number of roots significantly greater than those of the controls. When the 100 p.p.m. oestrone treatment was used only the 1000 p.p.m. concentration of indolylbutyric acid showed a significant increase in the number of roots over the controls, while the 500 and 1000 p.p.m. concentrations of oestrone completely offset any beneficial effect of indolylbutyric acid. Since none of the oestrone treatments in the absence of indolylbutyric acid had a significant effect, the apparent reduction in the number of roots on treatment with this chemical, as shown by the average values in Table II, must be attributed to the interaction with the plant hormone concentrations.

Data are given in Table IV for the interaction effects of indolylbutyric acid and oestrone on the length of roots per rooted cutting. In the absence of oestrone, indolylbutyric acid increased root length markedly, the 500 p.p.m. concentration was followed by a greater length of root than either the 100 or 1000 p.p.m. treatments and the results suggest that the highest level is past

TABLE IV

LENGTH OF ROOTS (MM.) PER ROOTED DORMANT *Lonicera tartarica* CUTTING ON TREATMENT WITH DUSTS CONTAINING INDOLYLBUTYRIC ACID AND OESTRONE

Data are means from 4 groups of 10 cuttings

Oestrone treatments, p.p.m.	Indolylbutyric acid treatments, p.p.m.			
	0	100	500	1000
0	72	162	469	432
100	89	221	247	286
500	59	125	254	179
1000	49	43	172	219

Necessary difference, 5% level, 24 mm.

the optimum. In the absence of indolylbutyric acid the 100 p.p.m. concentration of oestrone suggested an increase in root length; while neither the 500 nor the 1000 p.p.m. concentrations were significantly below the control, both were below the 100 p.p.m. concentration in respect to root lengths. In conjunction with 100 p.p.m. indolylbutyric acid the same concentration of oestrone increased root length, but the two higher concentrations reduced length markedly. The greatest reduction occurred at the 1000 p.p.m. concentration. In conjunction with 500 p.p.m. indolylbutyric acid, oestrone reduced length of roots, and the greatest reduction followed use of the highest concentration; in conjunction with 1000 p.p.m. indolylbutyric acid a somewhat similar condition was found in that all eostrone treatments depressed root length. However, while the 100 and 1000 p.p.m. oestrone treatments are significantly less damaging in conjunction with 1000 p.p.m. indolylbutyric acid than with the 500 p.p.m. concentration, the reverse is true of these combinations with the 500 p.p.m. oestrone concentration. In consequence, the depressive effects for oestrone treatments, as shown by the average values in Table II, may be attributed largely to its combination with the plant hormone indolylbutyric acid; the main effect was to decrease the root lengths that followed treatment with plant hormone; in one instance, at 100 p.p.m. of each in conjunction, an increase in root length was obtained.

It was pointed out that the interaction between indolylbutyric acid and oestrone on the number of cuttings rooted approached statistical significance. Examination of the data indicated that while oestrone treatment, averaged over all indolylbutyric acid treatments, reduced rooting as shown in Table II, this was to be attributed in part to its combination with the several indolylbutyric acid concentrations. In the absence of indolylbutyric acid, the three oestrone concentrations gave lower rooting than talc only, but none of them to a significant extent though depression of rooting increased with ascending oestrone concentration. Depression of rooting occurred to somewhat the same extent when oestrone was combined with the 100 and 1000 p.p.m.

concentrations of indolylbutyric acid, to a slightly lesser extent in combination with the 500 p.p.m. concentration. The cumulation of these effects accounts for the over-all depression of rooting shown by the oestrone treatments.

Conclusion

The results of this experiment are in general agreement with those described in an earlier communication in which *Lonicera tartarica* cuttings were treated with talc dusts containing three levels of both indolylbutyric acid and oestrone, each separately (2). In this former experiment cuttings were taken later in the season and after severe frosts, facts which, combined with absence of provision for study of the interaction of the chemicals, make close comparison of the results of the two experiments impossible. The present experiment has shown that the plant hormone has stimulated the rooting, number, and length of root per rooted cutting, the mean root length, and the green weight of leaf produced by the dormant cuttings. The marked stimulation of top growth by indolylbutyric acid treatments is particularly interesting. It may be pointed out that the numbers and lengths of roots and the weight of green leaf produced are relatively more sensitive methods of determining the effects of different hormone concentrations in dust than mere counts of the number of cuttings rooted. While there was no significant difference in percentage rooting at 100, 500, and 1000 p.p.m. indolylbutyric acid, the other responses showed the presence of marked effects attributable to concentration.

Oestrone treatments had a depressing effect throughout, though to a considerable extent the depressive tendency was related to the interaction with indolylbutyric acid. The results suggest that, in the concentration range investigated, oestrone tended to offset the beneficial effects of the plant growth stimulating chemical. However, the combination of 100 p.p.m. of each chemical had a beneficial effect on root length per rooted cutting, and it would appear that further experiments on oestrone and plant hormones in talc should deal with oestrone concentrations below 100 p.p.m.

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THE EFFECT OF CROP DEBRIS ON THE PATHOGENICITY OF CEREAL ROOT-ROTTING FUNGI[1]

By L. E. Tyner[2]

Abstract

The effect of wheat, oat, and barley straw, composted with soil, on the development of disease on the basal parts of wheat seedlings was studied in a series of greenhouse experiments during three seasons. The pathogens used were *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., and *Fusarium culmorum* (W. G. Sm.) Sacc. The wheat-straw composts were distinctly more favourable to the development of disease than the composts of either oat or barley straw. The least injury occurred on seedlings grown in composts of oat straw. It is suggested that the micro-organisms associated with the decomposition of oat straw bring about some degree of biological control of the plant pathogens also present.

Although the amount of straw applied sometimes influenced severity of disease, the effects were not consistent from planting to planting. Apparently the actual carbon to nitrogen ratio had less effect upon disease development than did the chemical nature of the straw.

The kind and amount of straw in the composts also influenced seedling vigour. This vigour was, in general, inversely proportional to the degree of infection.

The introduction of a short fallow period between plantings decreased infection somewhat and increased vigour.

The pathogenicity of the artificial inoculum added at the first planting was practically vitiated before the second planting. Subsequently, the infection ratings tended to increase and were about the same as those in the uninoculated series.

Introduction

It is common observation that the development and severity of the root-rot diseases of wheat caused by the soil-inhabiting pathogens, *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., and *Fusarium culmorum* (W. G. Sm.) Sacc., are favoured much more by certain crop sequences than by others. Recent experimental evidence has indicated that complex biological antagonisms and other obscure phenomena are, in part, responsible for the control indicated or for the lack of it, as the case may be.

Among the many factors that may favour or retard the degree of control resulting from a given crop, the kind and amount of crop residue returned to the soil should play an important role. On the other hand, the result in field soil, where the residue is probably subjected to a more complex biological environment, may be quite different than it is in artificial culture. Moreover, it is conceivable that the crop residue is not more important in determining the growth and virulence of the pathogens than the particular ecological conditions associated with a given crop or a bare summerfallow. Experimental evidence of the chain of events that occur in either case is almost wholly lacking.

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The object of the present study was to examine the differential effect in natural soil in the greenhouse of wheat straw, oat straw, and barley straw on the development of the root rots of wheat caused by *Ophiobolus graminis*, *H. sativum*, and *F. culmorum*.

Literature

The organic matter of the soil is a factor of importance in crop production. Schafer *et al.* (20) concluded that a carbon to nitrogen ratio in excess of 12 : 1 provided unfavourable conditions for crops. Greaves and Hirst (10) showed that it was possible to add all crop debris to the soil if the rotation chosen was effective in maintaining the nitrogen of the soil. Gray and McMaster (8) found that the activity of micro-organisms was dependent on the organic-matter relations in podsol soils. Waksman and Starkey (27), and Anderson (1) pointed out that the growing plant was quite unable to compete with micro-organisms in utilizing nitrogen when the cellulose content of the soil was high. When wheat straw was added to the soil, Murray (14) and Vandecaveye and Allen (22) found marked and immediate increases of bacteria, and, within a week, similar increases of fungi. The numbers of both groups fell off rapidly after 60 days at 20° C.

Any estimation of the rate and manner of decomposition must be made under conditions as nearly natural as possible. No significance may be attached to results obtained with one organism, for, as shown by Norman (15) and Waksman *et al.* (25), there is a far different effect from a mixed flora than is observed when only one organism is present. These mixed flora are of particular interest to the plant pathologist who is studying soil-inhabiting pathogens, because in the last analysis the virulence of a given pathogen or any increase or decrease therein must depend upon the associated microflora. Thus, Sanford (18) suggested that the control of potato scab in certain soils by incorporating green rye was attributable to the antibiotic effect, on *Actinomyces scabies*, of soil micro-organisms whose development was stimulated by the presence of green manure. The frequently observed (3, 4, 6, 7, 9, 16, 17), rapid diminution in the pathogenicity of *O. graminis*, *H. sativum*, or *F. culmorum*, added to soil in the presence of excess organic matter, is likewise probably attributable to the antagonism and competition of other soil-inhabiting micro-organisms stimulated by the organic matter added.

Materials and Methods

The experiments reported here were started in the greenhouse in the fall of 1935. Straw of wheat, oats, and barley was secured after harvest from contiguous plantings and reduced to the fineness of chop. The ground straw was then incorporated with a 3 : 1 soil-sand mixture in the proportions of 1.6, 3.1, and 4.7% by weight, and placed in six-inch, unglazed flower pots. The pots containing the mixtures indicated, and also a control lot filled with soil only, were placed on greenhouse benches for 17 days, during which they were kept moist. At the end of this period 20 surface-disinfected seeds of

Marquis wheat, previously graded for size, were sown. All treatments were replicated four times. At the beginning of the experiments, soil-grown inoculum of *O. graminis*, *H. sativum*, or *F. culmorum* was added with the seed in ten-gram quantities. In preparing the soil inoculum, all but a minimum of organic matter was excluded. In the case of *O. graminis*, air-dry soil was mixed with 5% corn meal by weight, 250 cc. of potato water from 125 gm. potatoes per litre of water, and 20 gm. of dextrose per kg. of dry soil. In preparing the inoculum of *H. sativum* and *F. culmorum* only corn meal was added to the soil. These media were steam sterilized, then inoculated and incubated for three weeks at room temperature.

The position of the pots in each replicate was changed weekly to eliminate local differences in temperature. A heavy wire screen was placed beneath the pots to assist aeration and hinder contamination from below by capillary suction. When the pots were being watered, care was taken to prevent run off. At the end of 40 days the severity of disease on each seedling was estimated numerically in a range of 0 to 10. Parallel series of oat and barley seedlings were planted, but for the sake of brevity the results are not reported in this paper.

The 1935–1936 Experiment

First Three Plantings

During the first three plantings of this experiment additional inoculum was not applied for the second and third crop. The pots were simply replanted immediately after each crop of wheat seedlings was removed.

In the first planting there was slightly more disease in the *H. sativum* series than in the corresponding *F. culmorum* series. In the *O. graminis* series it was about half as severe as in the *H. sativum* series, but approximately twice as great as that in the uninoculated soil-straw controls. A relatively low percentage of plants survived in the *H. sativum* and *F. culmorum* series, which indicated that both pathogens were quite active under the conditions. The survival value in the *O. graminis* series was slightly less than in the uninoculated control. Apparently there was no consistent effect of the kind or amount of straw on the disease ratings in any of the series (Fig. 1 C).

In the second planting, disease in the *H. sativum* and *F. culmorum* series was only about one-third as severe as in the first planting, and in the *O. graminis* series about half as great. The amount in the uninoculated controls was approximately the same as in the first planting and not significantly different from that in the inoculated series. The number of surviving plants was normal in all series, which indicates that all three pathogens were less active than in the first planting. There was a tendency for more disease in the wheat-straw compost than in the other composts, and also possibly a slight tendency for more disease in the highest concentration of straw (Fig. 1 C).

In the third planting the disease was approximately the same as in the preceding planting in all series, with the exception of the *H. sativum* series, where it was somewhat greater. With regard to the *O. graminis* series, it is known

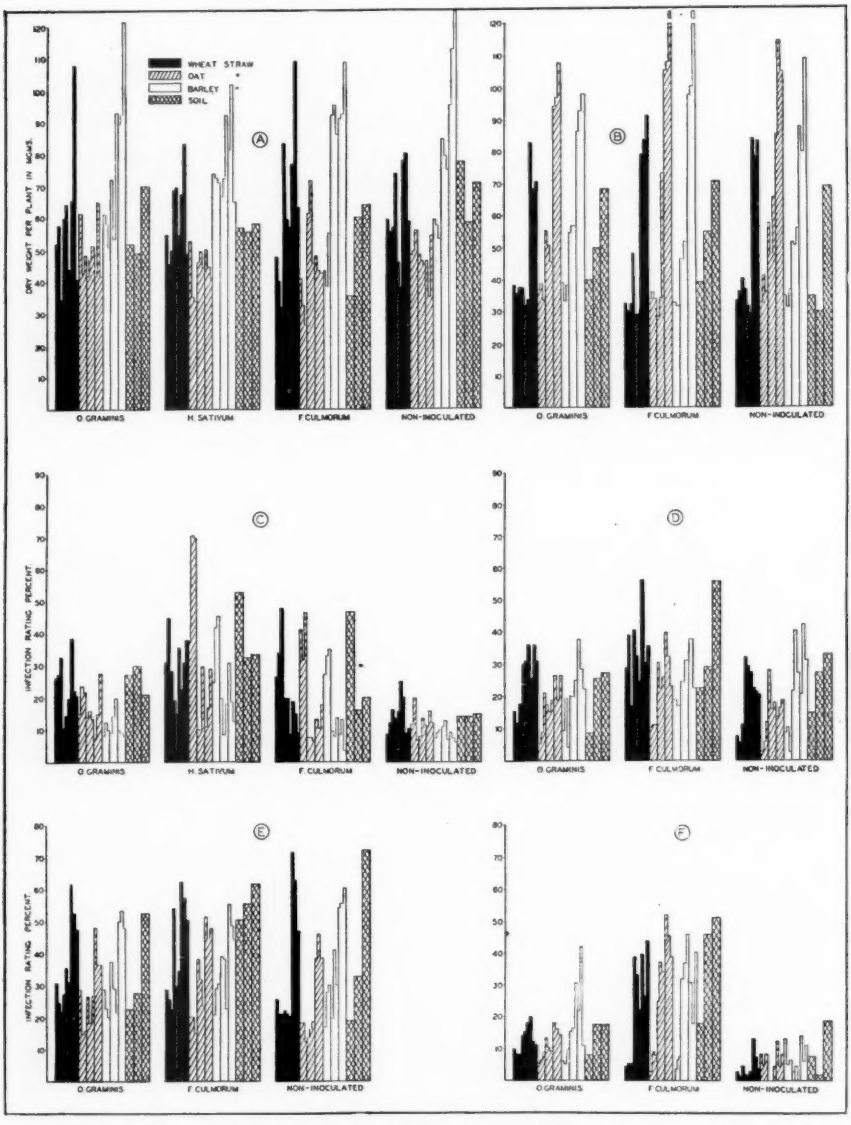

FIG. 1. *Effect of wheat, oat, and barley straw in three different amounts (1.6, 3.1, and 4.7%) in inoculated soil-straw composts, on the vigour and disease of wheat seedlings. These effects are illustrated from three plantings of three separate experiments, as follows:* A *and* C, *dry weight and disease, respectively, in plantings 1, 2, and 3, 1935 experiment.* B *and* D, *dry weight and disease, respectively, in plantings 1, 3, and 5, 1936 experiment.* E *and* F, *disease in unsterilized and sterilized soil series, respectively, in plantings 4, 5, and 6, 1936 experiment. Each large column represents a planting in the order named, and the three subdivisions of each column the variations for each straw concentration in order mentioned.*

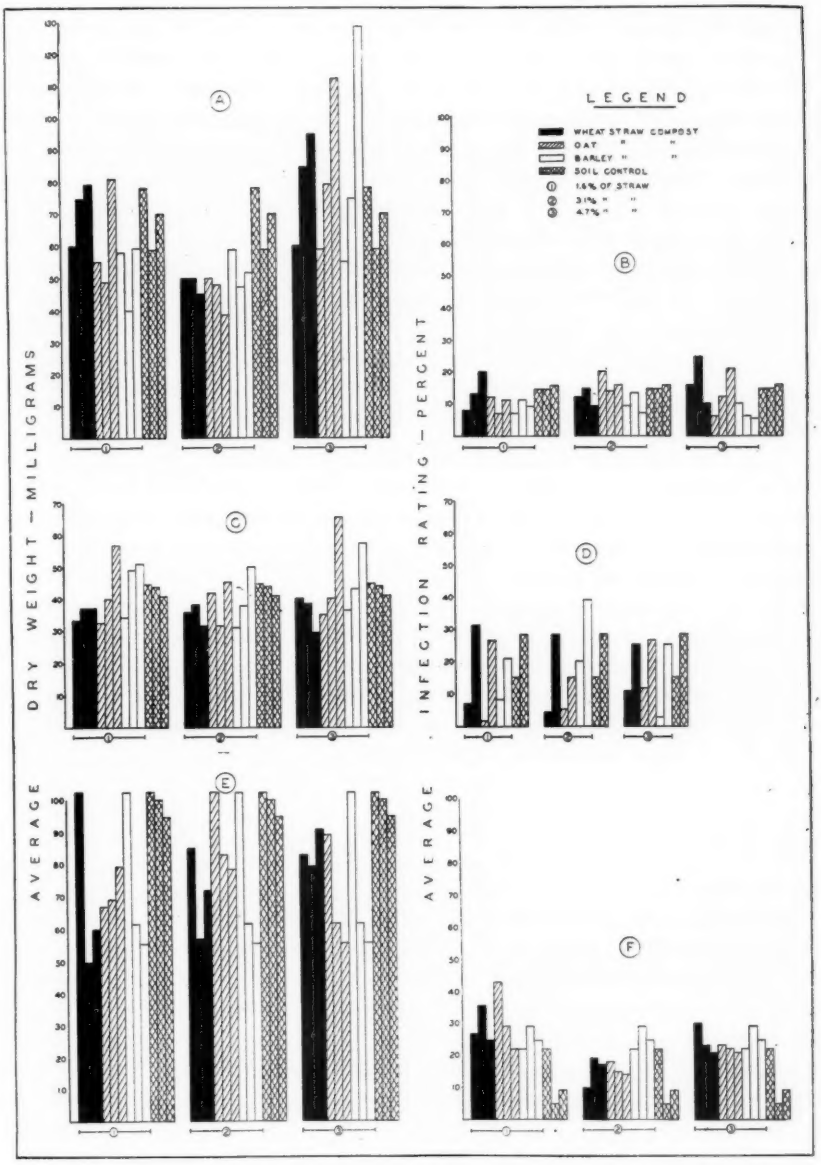

FIG. 2. *Effect of wheat, oat, and barley straw in three different amounts (1.6, 3.1, 4.7%) in uninoculated soil-straw composts, on vigour and disease of wheat seedlings. These effects are illustrated for the first, second, and third plantings of three separate experiments, namely, 1935,* A *and* B*; 1936,* C *and* D*; and 1938,* E *and* F*. Each column represents a planting.*

that both the virulence and the persistence of the inoculum of this pathogen are quickly vitiated when added to either sterilized or natural soil (2, 16, 19). Therefore, the disease observed in this series was probably caused either by *H. sativum* or *Fusarium* spp., or both. In this connection it is significant that, with the exception of the wheat-straw compost, there was approximately the same amount of disease in the *O. graminis* series as in the uninoculated control. Also, there was again a tendency for more disease in the wheat-straw compost than in the corresponding oat and barley composts. The higher concentration of wheat straw favoured more disease in the *H. sativum* series, but less in the other series. With oat straw, there was an increase in disease at the higher concentrations in all series, but in the barley compost there was a decrease (Figs. 1 C, 2 B). The number of surviving plants was approximately normal in the barley compost in all series, but quite low in the oat compost, and in the two highest concentrations of wheat straw in all inoculated series. The reasons for this are at present obscure.

With regard to plant vigour in the first three plantings, there was, with a few exceptions, a close relation between the amount of disease and the dry weight of plants. An increase in infection rating was correlated with a decrease in dry weight of the plants. The relative fertilizing values of the three kinds of straw composts are indicated by the dry weight of the plants in the control series. For example, in the first planting growth was inhibited in all composts to about the same extent. However, in the second and third crops the growth was increased on the barley-straw compost, but decidedly inhibited in the oat-straw compost. In the former case, growth increased with the concentration of straw, but in the latter all concentrations were equally inhibitory. Growth on wheat straw in the second planting was stimulated in the lowest concentration and inhibited in the higher ones. In the third crop the highest concentration inhibited growth, whereas the two lower ones stimulated it (Figs. 1 A, 2 A).

Fourth Planting

When the third planting was harvested, the experiment was modified in certain particulars. The pots in the *F. culmorum* series were treated with a 1 : 500 solution of mercuric chloride at the rate of 200 cc. per pot, while the pots of the uninoculated control series were steam sterilized. The two series originally inoculated with *O. graminis* and *H. sativum* were continued. In addition, all pots were allowed to remain fallow for two weeks. Further, the nitrate nitrogen was determined in the various 4.7% straw composts of the *O. graminis* series. For this the Spurway (21) spot-plate method was used. Tests were made from samples taken as soon as the third planting was harvested and again just before planting at the end of the fallow period. The nitrate nitrogen is listed below in parts per million of soil for each compost and the soil control, at the beginning and end of the test, respectively:

Wheat straw	Trace	9.25
Oat straw	0	Trace
Barley straw	Trace	16.6
Soil control	0	5.43

The foregoing data are of special interest in comparing the vigour of the seedlings of wheat on composts of wheat, oat, and barley straw in the third and fourth plantings. The test indicates that the available nitrate nitrogen was deficient at the end of the third planting.

The disease rating on wheat in the *O. graminis*, *H. sativum*, and *F. culmorum* series was essentially the same as in the third planting. Apparently neither the short fallow period to which all the series were subjected nor the treatment with mercuric chloride solution given the *F. culmorum* series, increased or decreased the disease. In general, considerably more disease continued to develop on the wheat-straw composts than on those of oat or barley straw. In view of the general increase observed in nitrate nitrogen during the fallow period, it would appear that this factor did not affect the level of disease. The plants in the steam-sterilized series were practically clean. Further, more plants survived than in the third planting. Possibly this was related to the general increase in fertility during the fallow period.

Plant vigour was greatly increased in all composts and soils in both the unsterilized and steam sterilized series. In the unsterilized series, the plants from the barley-straw composts were more vigorous than those from the corresponding wheat-straw composts, and those from the oat-straw composts were very much less vigorous, or, in general, about the same as those from the soil control. All steam-sterilized straw composts, and also the sterilized soil control, produced much more vigorous plants than the corresponding unsterilized units, and it is particularly significant that the yield on the oat-straw composts was somewhat greater than it was on either of the other two composts.

The fact that steam-sterilized oat-straw compost indicated greater fertility than those of wheat or barley straw under identical treatment suggests that the potential plant nutrients of the former had been from the beginning as great as in the latter composts, but that obscure biological processes associated with the decomposition and liberation of plant nutrients had been materially different in each case. The data on plant vigour and disease are given in Table I.

Fifth and Sixth Plantings

The main object in the fifth planting was to study the effect of fallow on disease and plant vigour. At the conclusion of the fourth planting the composts containing the three different percentages of each kind of straw were mixed to give for each an average of approximately 3% of straw, as based on the original composts. The procedure was followed for the two series originally inoculated with *O. graminis* and *H. sativum*, and the uninoculated series, which had been steam sterilized in the fourth planting. The *F. culmorum*

TABLE I

EFFECT OF VARIOUS STRAW-SOIL COMPOSTS ON VIRULENCE OF THREE ROOT-ROTTING FUNGI AND ON PLANT VIGOUR IN FOURTH CROP OF WHEAT SEEDLINGS FROM ORIGINAL INOCULATION OF COMPOSTS

Compost	Per cent	Average infection, %	Average height, cm.	Average dry weight, mg.
O. graminis				
Wheat	1.6	29	31	101
	3.1	23	38	168
	4.7	15	39	131
Oat	1.6	4	25	65
	3.1	13	31	80
	4.7	2	26	71
Barley	1.6	10	38	131
	3.1	4	36	128
	4.7	9	40	143
(Soil only)		16	36	100
H. sativum				
Wheat	1.6	37	36	112
	3.1	23	39	126
	4.7	19	32	90
Oat	1.6	13	34	110
	3.1	13	30	92
	4.7	9	26	67
Barley	1.6	30	32	98
	3.1	12	36	142
	4.7	8	40	146
(Soil only)		37	33	90
*F. culmorum**				
Wheat	1.6	22	38	116
	3.1	10	40	130
	4.7	17	37	104
Oat	1.6	4	38	107
	3.1	3	34	82
	4.7	4	36	103
Barley	1.6	7	39	119
	3.1	5	41	133
	4.7	2	42	141
(Soil only)		25	33	68
Uninoculated Control†				
Wheat	1.6	4	41	171
	3.1	2	38	166
	4.7	0	47	201
Oat	1.6	0	43	203
	3.1	0	46	230
	4.7	0	44	208
Barley	1.6	0	44	190
	3.1	1	43	175
	4.7	1	43	219
(Soil only)		1	41	163

* *Soil drenched with 200 cc. of 1 : 500 mercuric chloride solution per pot.*
† *Steam sterilized.*

series was discontinued. Thus, from each of the three kinds of straw composts in each of the three series indicated, there were prepared 20 pots for planting. Beginning May 26, and each week thereafter for four weeks, four pots of each straw compost from the *O. graminis* and *H. sativum* series, respectively, were inoculated with the pathogens and, with an equal number from the control series, planted to wheat. The remaining pots were thus in a state of fallow for one, two, three, or four weeks, as the case may be. The usual notes on disease and plant vigour, which were taken on the first four of the five plantings at the end of 35 days, are listed in Table II.

TABLE II

EFFECT OF FALLOWING VARIOUS STRAW-SOIL COMPOSTS FOR ONE, TWO, THREE, OR FOUR WEEKS ON PATHOGENICITY OF TWO ROOT-ROTTING FUNGI TO WHEAT SEEDLINGS

Compost	Weekly average per plant											
	Infection, %				Height, cm.				Dry weight, mg.			
	1	2	3	4	1	2	3	4	1	2	3	4
O. graminis												
Wheat	38	29	32	33	26	26	26	28	86	81	86	82
Oat	10	5	8	5	25	25	29	30	52	61	75	72
Barley	27	22	17	19	26	27	30	32	79	88	69	91
(Soil only)	32	25	30	30	23	26	22	23	51	70	50	47
H. sativum												
Wheat	33	31	30	29	27	28	31	30	76	87	73	89
Oat	31	21	17	15	23	25	28	32	56	87	74	110
Barley	34	27	28		28	28	29		81	93	89	
(Soil only)	34	29	25	26	22	23	28	24	46	70	94	44
Uninoculated												
Wheat	12	4	5	5	34	32	35	39	135	128	148	149
Oat	10	1	3	3	24	27	27	29	68	87	73	77
Barley	5	2	2	4	32	35	34	35	149	128	135	134
(Soil only)	13	10	12	16	33	30	29	33	140	99	90	101

Analyses for nitrate nitrogen in each kind of compost and soil in the *O. graminis* series were made before each planting. The phenol-disulphonic-acid colorimetric method was used. The results are listed in Table III. The first analysis revealed only a trace of nitrate nitrogen, but during the next week there was a relatively large increase. This increase was greatest with the wheat straw, but less marked in the barley and oat straw. The nitrate nitrogen in the soil control was much less in all succeeding tests than in the composts. Although the general order of increase mentioned was not maintained consistently in subsequent tests, more nitrates were found in both the

TABLE III

NITRATE NITROGEN* (P.P.M.) IN VARIOUS STRAW-SOIL COMPOSTS AS AFFECTED BY PERIOD OF FALLOW

Compost	Fallow period in days				
	0	7	14	21	28
Wheat	Trace	3.74	4.03	3.0	5.25
Oat	Trace	1.75	5.0	Trace	1.92
Barley	Trace	2.65	5.21	3.9	5.65
(Soil only)	Trace	0.75	1.38	Trace	1.62

* *The phenol-disulphonic acid method was used.*

wheat- and the barley-straw composts than in the oat-straw compost. In the latter case it was only slightly greater than in the soil control.

The disease on all weekly plantings was approximately the same, the only differences being with respect to the different straw composts. The seedlings on the oat-straw compost had consistently less disease than those on the other composts, or those on the inoculated soil control. This reduction appears to be related in some way with the associated microflora in the oat-straw compost. At any rate, it would seem that it is not a result of the low fertility indicated by the nitrate analysis, because disease was considerably more severe on the inoculated soil control which contained even less nitrate nitrogen than the oat-straw compost.

With regard to plant vigour, there was a definite tendency for an increase on all composts in each of the four plantings. There was no marked change in the soil control. Vigour of the seedlings on the oat-straw composts and the soil control series was less than on the wheat and barley composts. The only reason apparent from the data is that where an increase in vigour occurred in the two inoculated series it was related to an increase in nitrates.

Data on the seedlings in the uninoculated series, which had been steam sterilized in the preceding experiment, are of special interest, because they confirm the results obtained from the two inoculated series just discussed. For example, vigour was again decidedly less on the oat-straw composts than on either the wheat-straw or barley-straw composts, or even on the soil control, despite the fact that, in the preceding planting, growth on this oat-straw compost had exceeded that on either of the other two composts.

The effect of fallow was observed again in the sixth planting. Two weeks after harvest of the seedlings, tests were made by the Spurway method of the active nitrate nitrogen, potassium, and phosphorus in the various units of the experiment (Table IV).

In general, the soil controls in both inoculated and uninoculated (steam sterilized at fourth planting) series indicated less nitrogen, phosphorus, and potassium than any of the straw composts. The nitrate nitrogen was highest in the wheat straw composts, but it was approximately as low in the oat compost as in the soil control.

TABLE IV

NITRATE NITROGEN, POTASSIUM, AND PHOSPHORUS* IN VARIOUS STRAW-SOIL COMPOSTS TWO WEEKS AFTER HARVESTING THE SIXTH SUCCESSIVE CROP OF WHEAT SEEDLINGS

Compost	Treatment	Nitrate nitrogen, p.p.m.	Potassium, p.p.m.	Phosphorus, p.p.m.
Soil	*O. graminis*	Trace	5	0.5–1
	H. sativum	2	5	0.5–1
	Uninoculated	2–5	0–5	0.5–1
Wheat	*O. graminis*	0–2	5	0.5–1
	H. sativum	5–10	10	1–2.5
	Uninoculated	10–25	10	1–2.5
Oat	*O. graminis*	0–2	5	0.5–1
	H. sativum	5	10	0.5–1
	Uninoculated	Trace	5–10	1–2.5
Barley	*O. graminis*	2	5–10	1–2.5
	H. sativum	2	10	1–2.5
	Uninoculated	2–5	0–5	1–2.5

* *As indicated by the Spurway practical soil testing method.*

Nitrates were highest in the uninoculated pots containing wheat straw, barley straw, or soil only. Obviously the store of nitrate nitrogen released by sterilization had been maintained, at least in part, in these cases. However, nitrates were lowest in the uninoculated pots containing oat straw. This difference affords a reasonable explanation of the much reduced vigour of plants grown on the oat-straw composts in the fifth and sixth plantings. It is not known why the fertility of the oat-straw composts should be so much reduced, but a possible explanation is that it is related to differences in the microbiological conditions in the presence of the various straws. No noteworthy trends in the occurrence of phosphorus or potassium were apparent for any of the straw composts from these tests.

THE 1936–1937 EXPERIMENT

A new experiment, designed on the same general lines as that begun in the fall of 1935, was started in the fall of 1936. Uniformly mature straw of wheat, oats, and barley from a different locality was secured for making the various composts, and the same percentages of straw, viz., 1.3, 3.1, and 4.7%, were added to soil as in the previous experiment. The 4.7% series of barley straw was omitted from the *H. sativum* and *F. culmorum* series on account of insufficient material. The composts were originally inoculated with subcultures of the same strains of *O. graminis*, *H. sativum*, or *F. culmorum* that were used in the 1935 experiment. The usual data were recorded at the end of 40 days and the pots were replanted immediately.

First, Second, and Third Plantings

The results on wheat seedlings for the first three plantings are illustrated in Figs. 1 B and D, and 2 C and D.

Only a slight infection developed in the first planting. In fact, with the exception of the *F. culmorum* series, it was very little greater than in the uninoculated series. A light infection developed again in the second planting, but in the third one a moderate infection developed in all series, regardless of whether they had been previously inoculated or not. Apparently the disease was not caused by the artificially added pathogens. Decidedly less infection occurred on seedlings grown in the oat-straw composts than on the wheat- and barley-straw composts.

All composts appeared equally favourable to plant vigour in the first planting, but in the second and third plantings the wheat-straw composts were definitely deleterious, and this effect was most pronounced in the 4.7% units. In general, barley straw was more favourable than oat straw, but both were more favourable than the soil control. The height of the plants often varied greatly from one planting to another, according to the seasonal intensity of light, and, therefore, only variations in height in each planting are comparable. For this reason, dry weight indicated relative vigour more accurately than plant height.

Fourth Planting

It will be recalled that in the fourth planting of the 1935 experiment the effect of steam sterilization was studied by making certain adjustments. This time the *H. sativum* series was discarded, but the *O. graminis*, *F. culmorum*, and uninoculated series were continued. Half the pots in each series were steam sterilized, and these, with the corresponding unsterilized units, were reinoculated with *O. graminis* or *F. culmorum* as required. For reinoculation, 10 gm. of soil-grown inoculum of the pathogen was added with the seed at time of planting. The data on infection ratings are given in Fig. 1 E and F.

In the inoculated series of the unsterilized section, the amount of disease was practically the same as in the third planting, and was not significantly greater than in the uninoculated control. Also, plant vigour was relatively the same as in the third planting. The seedlings grew best on the oat-straw composts, and they were least vigorous on the wheat straw. Vigour increased with increasing amounts of oat or barley straw in the composts, but tended to decrease as the proportion of wheat straw increased. The least growth occurred on the soil controls. Obviously all three kinds of straw compost were more fertile than the soil alone.

In the steam sterilized, reinoculated series, however, disease was practically absent. Apparently the amount of inoculum applied was insufficient to be effective under these conditions. Evidence on this point will be found in the next planting. Plant vigour was greatly stimulated by sterilization. The greatest growth of seedlings occurred on the composts containing the least straw, which is opposite to what occurred in the unsterilized series. Wheat straw in the 4.7% proportion was the least stimulatory. Again, as in the unsterilized section growth was greater in most cases on all the straw composts than on soil alone.

Fifth Planting

The purpose of this planting was to obtain further data on the effect of steam sterilization of the composts on disease and plant vigour, and to study the influence of a two-week fallow period.

After taking up the seedlings from the fourth planting all pots were fallowed for two weeks. Half the pots were then again steam sterilized. The inoculated series was reinoculated this time with 20 gm. of the soil inoculum of *O. graminis* or *F. culmorum*. The data on disease and dry weight are illustrated in Fig. 1 B, D, E, and F.

In the unsterilized set, inoculation by either organism caused significant infection only in the wheat-straw composts of both series, and in the soil control only in the *F. culmorum* series. There was less disease on seedlings grown in oat-straw composts than in those of wheat or barley straw, or in the soil control of all three series. There was no definite relation between disease and amount of straw used in the three series, but quite marked differences were apparent in individual cases. There were 16% fewer surviving plants in the *F. culmorum* series than in the *O. graminis* series.

There was a marked increase in vigour over the fourth planting. This, undoubtedly, was caused by the period of fallow. Dry weight was greatest on the composts of oats and barley straw but was reduced by more than 25% when wheat straw was used. All three kinds of compost supported much more growth than the soil only.

In the sterilized units, the artificial inoculation by both *O. graminis* and *F. culmorum* was fairly effective. *F. culmorum* caused over twice as much disease as the former organism, and, in addition, decreased the number of surviving plants by 15% more than *O. graminis*. In both inoculated series, disease was about the same on seedlings grown in oat-straw and wheat-straw composts, and approximately 19% higher on composts of barley straw. The amount of straw had no consistent effect on disease in this test.

The plants were slightly more vigorous than in the fourth planting. This was probably a result of the period of fallow given. Dry weights were about 10% less in the *F. culmorum* series than in either the *O. graminis* or the uninoculated series. In the latter series the most vigorous plants were on the oat-straw compost and the least vigorous ones on barley straw. The differences in growth between the sterilized and unsterilized series were greater on the wheat-straw composts than on the other two composts. Vigour was not consistently affected by varying the amount of straw, but was always greater on the composts than in the soil control.

Sixth Planting

The purpose of this planting was to observe further the effect of sterilization on disease and plant vigour, and to note the effect of continued cropping on the increase of disease on plants in composts never artificially inoculated.

Immediately after taking up the fifth planting, the pots that had been steam sterilized in the two previous plantings were again sterilized, and the

corresponding section that had never been sterilized was continued as before. To the pots of the inoculated series in both sections was again added 20 gm. of inoculum of *O. graminis* or *F. culmorum*, as required. All pots were reseeded to wheat. The data on disease are illustrated in Fig. 1 E and F.

The severity of the disease in the unsterilized section of the *F. culmorum*, *O. graminis*, and uninoculated series, increased over that in the corresponding series of the fifth planting by 41, 80, and 135%, respectively. Also, the infection rating was as high in the artificially inoculated series as in the uninoculated series, which, in view of the almost complete absence of disease in the previous planting, is an interesting, but by no means unusual situation in experiments of this sort. Disease was slightly decreased by the oat-straw composts, as indicated by the average rating of 43% in comparison with 53% and 57% for the barley and wheat-straw composts, respectively.

Plant vigour averaged approximately 45% less than in the fifth planting. Although some of this was due to the increase in disease, most of the difference was probably traceable to the fallow period prior to the fifth planting. The barley-straw composts favoured growth slightly more than either of the others. Also, growth appeared slightly increased at the higher concentrations of straw in the wheat and barley composts. There was no difference in the oat-straw composts.

With regard to the sterilized series, both *O. graminis* and *F. culmorum* were considerably more virulent than in the unsterilized series, and the latter pathogen caused twice as much disease as the former. The oat-straw composts favoured disease more than either the barley or the wheat composts, which is contrary to the results consistently obtained in the unsterilized soil in all previous tests. Although neither the kind nor the amount of straw in the composts indicated any very marked differential effect on plant vigour, the fertilizing value of it was evident in comparison with the soil control.

The 1938–1939 Experiment

The work was repeated to obtain further evidence regarding the effect of wheat-, oat-, and barley-straw composts on *H. sativum*, *F. culmorum*, and *O. graminis*.

The composts were each prepared as in previous experiments. Only two proportions of straw were continued, namely, 1.6 and 4.7%. The pots were inoculated only once, just before seeding in the first planting. Two kinds of soil controls were used, namely, one inoculated at the beginning and carried throughout all plantings, and another freshly prepared and inoculated for each succeeding planting. The usual parallel inoculated and uninoculated portions of each compost and soil control series were maintained.

In general the results of this experiment confirmed those of the first and second experiments. For example, the severity of disease in the inoculated and uninoculated series was not significantly different. The average disease ratings in the inoculated series on the oat-, barley-, and wheat-straw composts

TABLE V

THE EFFECT OF VARIOUS STRAW-SOIL COMPOSTS ON THE VIRULENCE OF THREE ROOT-ROTTING FUNGI AND ON PLANT VIGOUR DURING FIVE SUCCESSIVE CROPS OF WHEAT SEEDLINGS

Compost	Per cent	Av. infection, %					Av. dry weight, mg.				
		1	2	3	4	5	1	2	3	4	5
O. graminis											
Wheat	1.6	24	33	27	35	23	125	76	63	50	44
	4.7	33	23	28	29	27	71	101	82	67	60
Oat	1.6	19	35	26	37	26	100	83	72	53	47
	4.7	17	25	31	28	9	96	101	73	52	46
Barley	1.6	25	22	26	35	16	103	88	62	48	42
	4.7	20	26	19	34	22	103	96	75	60	47
Soil*		38	34	36	52	29	92	70	60	43	40
Soil†			10	53	18	3		101	63	65	70
H. sativum											
Wheat	1.6	35	39	32	44	36	94	82	61	45	43
	4.7	31	30	28	40	34	73	85	79	68	52
Oat	1.6	30	44	32	37	30	85	73	63	46	46
	4.7	22	30	33	40	21	91	103	72	53	46
Barley	1.6	44	41	29	45	22	88	89	70	51	48
	4.7	26	28	31	47	18	99	108	78	57	47
Soil*		33	38	29	42	14	101	83	68	46	47
Soil†			31	24	24	19		101	99	97	66
F. culmorum											
Wheat	1.6	21	29	36	45	32	112	81	82	50	43
	4.7	31	22	33	37	25	80	88	85	60	58
Oat	1.6	16	21	25	32	14	74	92	89	53	47
	4.7	17	20	16	25	11	89	102	77	49	56
Barley	1.6	49	38	30	34	24	95	96	72	56	44
	4.7	27	35	29	35	20	107	105	83	60	50
Soil*		16	36	26	44	26	117	88	70	42	41
Soil†			6	11	22	4		97	82	68	57
Uninoculated											
Wheat	1.6	27	36	25	44	28	103	50	60	47	40
	4.7	43	29	22	29	22	67	69	79	63	45
Oat	1.6	10	19	17	26	16	85	57	72	45	43
	4.7	18	15	14	19	23	103	83	78	62	45
Barley	1.6	30	23	21	33	35	83	74	66	50	43
	4.7	23	22	19	30	18	99	79	91	46	44
Soil*		22	29	25	43	27	103	62	56	43	42
Soil†			5	9	10	5		100	95	67	70

* *Permanent control.*

† *Control freshly prepared for each planting.*

were 26, 30, and 30%, respectively, indicating that, during decomposition, wheat straw rather favours disease, and that decomposing oat straw tends to check it. Also, there was some evidence of a trend toward less disease at the 4.7% concentration of straw than at 1.6%. Finally, it was again evident that a relatively small amount (10 gm.) of inoculum added to a natural soil was, under the conditions of these experiments, usually only slightly effective on the first crop (Fig. 2 E and F, and Table V).

Discussion

In these experiments, where a small quantity of inoculum was added to the pots, disease development was relatively light on the first crop of seedlings and, as is usual, it was definitely less on the second and third crops. It was never as severe as that which usually occurs in a freshly steam-sterilized, inoculated soil. There was some tendency for an increase of disease beginning with the fourth crop from the originally inoculated series. This trend was not consistent. Also, beginning with the second planting, the increase of disease in the various control units, which were not artificially infested, was almost identical with that of the corresponding units originally infested artificially. Thus, it would appear that the increase during a year or so of a pathogen in a given soil, whether in field or greenhouse, would depend mostly on the initial natural infestation, assuming, of course that other conditions were favourable. In other words, if the bio–physico-chemical state of a given soil were not favourable for a natural increase of the pathogen, a large quantity of artificial inoculum would be no more effective than a relatively small amount.

There was significantly less disease on the seedlings grown on oat-straw compost than on those from either of the other two composts. Also, in general, the composts of barley-straw seemed slightly less conducive to disease than those of wheat straw. The results mentioned may be best explained by assuming that the dominant microflora of the oat-straw composts were somewhat antagonistic to the pathogens, or characteristically different from that of the other composts (23).

There was some evidence that the concentration of straw in a compost affected the severity of disease, but this effect was not consistent enough in different plantings to warrant a conclusion. If each kind of straw has a characteristic microflora associated with its decomposition, the final effect on the pathogen might depend more on the degree of antagonism present than on certain differences in its concentration. This theory is supported by the conclusion of Waksman and Hutchings (26) that wheat straw is more resistant to decomposition than oat straw, and by the work of Martin (12) and Millar *et al.* (13), who concluded that the maximum rate of decomposition of a given plant material depends upon the presence of a specific microflora.

This study provides some interesting comparisons on the rate of increase of the three pathogens in the various inoculated and uninoculated series. First, *O. graminis* produced disease only on the first crop following inoculation,

and then seemed to disappear completely, regardless of the presence or absence of decomposing wheat, barley, or oat straw. In contrast is the interesting fact that, starting with a slight natural infestation of a summerfallowed field, this pathogen is known to increase sufficiently to cause fairly severe damage to the second and third successive crops of wheat. Apparently the conditions necessary for the rapid increase of *O. graminis* are provided by successive crops of wheat in the field, but not in pot culture in the greenhouse.

H. sativum and *F. culmorum* are similar to *O. graminis* with respect to rapid vitiation of artificial inoculum, whether added to the soil in either field or greenhouse culture, but different in that both tend to increase in greenhouse culture with successive cropping to wheat. Such increase does not seem to depend upon the original inoculum added, nor is it appreciably affected by the presence or absence of decaying wheat, oat, or barley straw in the soil.

Plant vigour was used as an aid in estimating the relative effect of the different kinds of straw composts on disease. The height of the plants was not considered as reliable an index as dry weight, for during the winter months, despite supplementary artificial illumination, the plants were tall in relation to their weight, whereas during autumn and spring, under normal sunlight, the relation between height and weight was close. Growth was significantly suppressed in the first planting on the three kinds of composts. However, in the second planting this inhibition was replaced by a stimulatory effect, which usually reached a maximum by the fourth planting. After this time plant vigour gradually decreased, even though light conditions were more favourable. Evidently this latter decrease in vigour was due to natural reduction of available plant nutrients.

Plants grown on wheat-straw composts were, in general, the least vigorous throughout the study. The most vigorous plants were produced on the barley-straw composts. Growth on oat-straw composts varied. In 1935, when the straw was immature, the plants were quite definitely lacking in vigour and rather chlorotic, but in 1936 and in 1938 they were better than those on wheat straw, and only slightly less vigorous than the seedlings on the barley-straw composts. With regard to the relation of the amount of straw in a compost to vigour, it was generally apparent that when a particular kind of straw reduced growth, it was directly proportional to the amount added. Thus, in the case of wheat straw, least growth occurred at the highest concentration, while for barley straw the opposite was true. In order to explain the situation just mentioned, one must assume that it was due to one or more factors, namely, the chemical composition of the straw, the release of toxins, rate of decomposition, the kinds of micro-organisms involved, and the C/N ratio established in the different composts.

With regard to toxins, Collison and Conn (5) found that products toxic to plants were released from decomposing wheat or barley straw. Similar evidence is supplied by Waksman (24). As for the effect of the chemical constitution of certain crop debris on decomposition, Hutchings and Martin (11) found that this was more important than the C/N ratio established in

the soil. Further, according to data secured by the present study, indications were that the difference in nitrate nitrogen content of the three kinds of straw were not directly proportional to the differences observed in plant vigour. However, one might assume that the complete chemical complex of a given organic material is often apt to be of greater importance to plant growth than a single chemical constituent of it.

Thus, concluding this discussion, it appears that the observed differences in persistence and virulence of the pathogen, and in plant vigour, were caused mainly by differences in the microflora associated with the decomposition of each kind of compost.

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THE INTERACTION OF HIGHER PLANTS AND SOIL MICRO-ORGANISMS

I. MICROBIAL POPULATION OF RHIZOSPHERE OF SEEDLINGS OF CERTAIN CULTIVATED PLANTS[1]

By M. I. Timonin[2]

Abstract

The microbial population in the rhizosphere of wheat, oats, alfalfa, and peas was studied and the relative abundance of different types of micro-organisms recorded. By means of the plating method it was found that bacteria and actinomycetes were 7 to 71 times greater in the rhizosphere than in the soil distant from the roots, whereas fungi were but 0.75 to 3.1 times more numerous.

Different varieties of plants affected the activity of the various groups of soil micro-organisms differently. Thus fungi were more numerous in the rhizosphere of oats, and bacteria in the rhizosphere of alfalfa. Seventeen genera of fungi were represented in isolates from the rhizosphere of seedlings and the soil distant from the roots. However, no marked difference was observed in the types isolated from the rhizosphere of different varieties of seedlings. A fungus isolated from the rhizosphere of alfalfa proved to be a new species and the genus *Spicularia* Persoon was amended to include it.

Introduction

The living part of the soil complex consists of immense numbers of micro-organisms and of roots of higher plants. These two domains of living organisms, using the organo-mineral part of the soil as the medium for their existence, are not only in direct contact but also in a state of continuous interaction. The portion of the soil in which this interaction takes place is called the rhizosphere. It is from the rhizosphere that higher plants obtain their necessary nutrients and it is into the rhizosphere that they excrete by-products of their growth. Owing to the physiological function of the roots, not only are the physical and chemical properties of the soil of the rhizosphere changed, but the microbial activities are greatly affected.

The immense microbial accumulations localized in the near vicinity of the roots may be directly (nodule bacteria, mycorrhizal fungi) or indirectly (e.g., nitrifiers, cellulose decomposers) beneficial to higher plants. However, it should be recognized that the development of different micro-flora in the rhizosphere may be injurious.

Plants in the seedling stage are very sensitive to environmental conditions including direct or indirect attack by soil micro-organisms. The interaction of soil micro-organisms and the seedlings of cultivated plants is therefore of great scientific as well as practical interest. The purpose of this and the following investigations is to throw some light on this interaction and the influence of environmental conditions on it.

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The work here reported was carried out at the New Jersey Agricultural Experiment Station and in the Division of Bacteriology and Dairy Research, Department of Agriculture, Ottawa. Contribution No. 65 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

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Historical Review

Considerable work on the influence of higher plants on the activity of soil micro-organisms has been recorded in the literature. It can be roughly subdivided into two groups as follows: *(a)* reports concerned with changes of microbial accumulations in cultivated soil owing to the influence of growing crops, and *(b)* reports chiefly concerned with the microbial population of the rhizosphere of cultivated plants.

Caron (3) in 1895 was the first to report that the microbial population of the soil is greatly influenced by the crop grown and the methods of cultivation of the soil. In the same type of soil he found four times as many bacteria under clover as under sugar beets. LeClair (16) and Greaves *et al.* (10) found more organisms in cultivated than in fallow soil. Greaves (9) reported more organisms in the soil under wheat than in the corresponding soil under alfalfa, though Creuzburg (4) reported results contrary to this. Stoklasa (27) obtained different numbers of soil organisms from soil under different crops. Furthermore, his results indicate a positive correlation between the numbers of bacteria in soil under different crops and the evolution of carbon dioxide.

In the middle of the nineteenth century it was already proved (13, 23) that plants through their root systems excrete certain chemical compounds into the surrounding soil. Taking this fact into consideration, Hiltner (11) claimed that the root excretions made the soil around the roots a very favourable medium for the activity of soil micro-organisms. He found greater microbial accumulations in the soil around the roots than elsewhere in the same soil, and to the portion of the soil influenced by the physiological function of the roots he applied the term "rhizosphere".

Hiltner's results concerning the abundance of micro-organisms in the rhizosphere were later confirmed by many workers (8, 12, 21, 22, 30). It has also been shown that the activity of micro-organisms is markedly affected by the degree of plant development. The maximum effect was noticed when the plant was in an advanced stage of vegetative development or in the flowering stage (26). Smith (24) reported stimulative effects of leguminous plants on the number of *B. radiobacter* in the third week after planting. Other workers also presented evidence that different plants exercise a different influence on the activity of soil micro-organisms (25). Krassilnikov *et al.* (14) presented evidence showing that different stages of plant development support different types of soil micro-organisms. Obrazcov (19), however, failed to note any difference in types of micro-organisms in the rhizosphere of different plants. Several other workers (15, 18, 25) reported on the interaction of higher plants and soil micro-organisms; but there is practically no information concerning the influence of the higher plants in the seedling stage on the soil micro-organisms. During the last three decades, the fungous flora of the soil has attracted the attention of several investigators (1, 2, 28, 31, 32). The subject has been studied from various points of view; however, there are practically no records concerning the influence of higher plants on the fungous flora of the rhizosphere.

Material and Methods

A Sassafras sandy loam soil of pH 5.6, and with 4.9% of organic matter, was passed through a $\frac{1}{8}$-in. mesh sieve and placed in the greenhouse in a wooden box, 5 by 2 by 1 ft. Seeds of Kota hard spring wheat, oats, alfalfa, and soybeans were planted. Each variety was sown in a separate row, allowing enough space that roots of individual plants as well as of different varieties would not overlap. Prior to sowing, seeds were surface sterilized in mercuric chloride 1 : 1,000 solution: oats for seven, wheat and peas for five, and alfalfa for two minutes. After sterilization the seeds were washed in sterilized tap water in five consecutive changes. The samples of seedlings and the soil (control) were taken for microbiological analyses 3, 6, 10, and 16 days after planting.

Samples of the soil from the rhizosphere of the seedlings were obtained as follows: the block of soil about $2\frac{1}{2}$ by $2\frac{1}{2}$ by 3 to 5 in. surrounding the seedling roots was cut out and placed in a large porcelain evaporating dish. Different numbers of seedlings were taken at different times of sampling. Thus three days after planting, 3 to 5 seedlings of wheat, oats, and peas, and 10 to 15 seedlings of alfalfa were obtained. In the laboratory the soil blocks were gently crushed with as little tearing of the roots as possible, the seedling carefully removed, and the roots gently shaken. The top at the crown or foot of the seedling was cut off and the roots with the soil attached deposited in a sterile tared Erlenmeyer flask. The flask and contents were weighed again and the weight of roots with the attached soil determined. Assuming that the weight of the attached soil was approximately one-half to one-third (depending on how much soil was attached to the roots) of the total weight, the dilutions for plating were prepared accordingly.

The dilution in the original flask was made 1 : 100 and was shaken by hand for five minutes. Subsequent dilutions were shaken for one minute. When the required dilutions were prepared, the roots were removed by means of forceps from the original flask, the water evaporated on a water bath, and the soil residue dried to constant weight in an oven at 105° C. Thus the weight of moisture-free rhizosphere soil was obtained so that dilution coefficients could be calculated. The dilutions of soil (control) were made on a 10 gm. basis.

The numbers of micro-organisms were estimated by the plating method, counts of suitable dilutions being made on four replicate plates and the average counts recorded. Waksman's (6) peptone-glucose acid agar (pH approx. 3.8 to 4.0) was used for fungi. The numbers of bacteria and actinomycetes were estimated on Waksman's sodium albuminate agar (6) by counting all colonies developing on the plate. All plates were incubated at 28° C., four days for fungi and ten for bacteria and actinomycetes.

For the systematic study of fungi one plate with good distribution of colonies was selected and each individual colony transferred to a slant of the same (non-acidified) medium.

Density of Microbial Population in the Rhizosphere

From the data presented in Tables I and II and in Figs. 1 and 2 it is quite evident that the numbers of microbial counts are larger, except in one case, in the rhizosphere of all four varieties of plants studied, than elsewhere in the same soil. The numerical difference in counts in the rhizosphere and in the soil distant from the roots is clearly shown in the rhizosphere-soil ratio. Counts of bacteria and actinomycetes are from 7 to 71 times greater than in the soil elsewhere. In the case of filamentous fungi, the difference is not as striking, the lowest ratio being 0.75 and the highest 3.1. The data also indicate that each of four varieties of plants influences to a different degree the relative abundance of the various groups of organisms in its rhizosphere. Comparing the counts of colonies obtained from the rhizosphere of the different varieties of the plants studied, oats showed lowest counts in the case of bacteria and actinomycetes, and highest in the case of filamentous fungi in all samples. Alfalfa, however, was higher in bacteria and somewhat intermediate in the case of fungi. As shown in Figs. 1 and 2, the data indicate in general an increase in counts with advancing development of the seedlings.

TABLE I

NUMBERS OF BACTERIA AND ACTINOMYCETES IN THE RHIZOSPHERE AND SOIL DISTANT FROM THE ROOT

Numbers expressed in millions per gram of moisture free soil

Plant	Sample from	1st Sample		2nd Sample		3rd Sample		4th Sample	
		Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio
Wheat	Rhizosphere	275.8	12.9	1110.2	49.3	360.7	16.1	523.3	19.1
	Soil	21.3		22.5		28.5		27.3	
Oats	Rhizosphere	230.2	11.9	180.1	8.5	210.5	7.7	312.4	12.8
	Soil	20.7		21.3		27.3		24.5	
Alfalfa	Rhizosphere	287.6	12.9	870.4	42.9	1150.4	46.8	1824.3	71.0
	Soil	22.3		20.4		24.6		25.7	
Peas	Rhizosphere	470.0	22.8	540.1	24.0	800.4	30.3	934.5	37.8
	Soil	20.6		22.5		26.4		24.7	
Average in soil		21.2		21.7		26.7		25.6	

$\frac{R}{S} = \frac{\text{Rhizosphere; soil near the roots}}{\text{Soil distant from the roots (control)}}$

TABLE II

NUMBER OF FILAMENTOUS FUNGI IN THE RHIZOSPHERE AND IN THE SOIL DISTANT FROM THE ROOTS

Numbers expressed per gram of moisture free soil

Plant	Sample from	1st Sampling		2nd Sampling		3rd Sampling		4th Sampling	
		Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio	Numbers	$\frac{R}{S}$ ratio
Wheat	Rhizosphere	190,000	1.1	241,000	1.3	320,000	1.9	256,000	1.4
	Soil	175,000		180,000		172,000		172,000	
Oats	Rhizosphere	210,000	1.1	320,000	1.8	415,000	2.5	570,000	3.1
	Soil	185,000		174,000		168,000		186,000	
Alfalfa	Rhizosphere	130,000	0.75	210,000	1.2	231,000	1.3	511,000	3.0
	Soil	174,000		176,000		176,000		169,000	
Peas	Rhizosphere	241,000	1.4	212,000	1.2	272,000	1.5	460,000	2.7
	Soil	169,000		172,000		173,000		173,000	
Average in soil		176,000		176,000		172,000		175,000	

$$\frac{R}{S} = \frac{\text{Numbers in rhizosphere}}{\text{Numbers in soil (control)}}$$

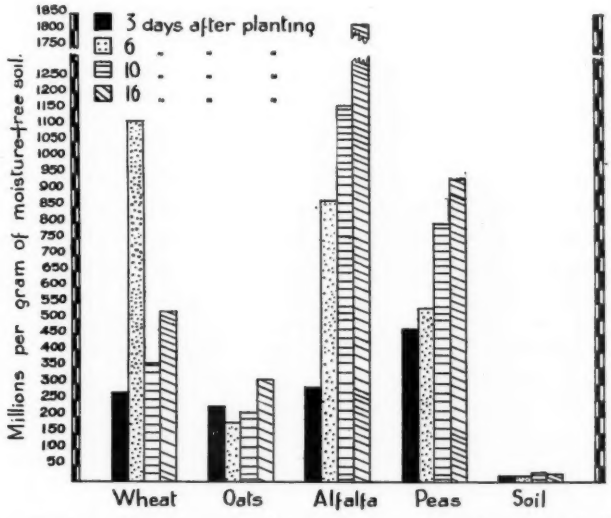

FIG. 1. *Numbers of bacteria and actinomycetes in the rhizosphere and soil distant from the roots.*

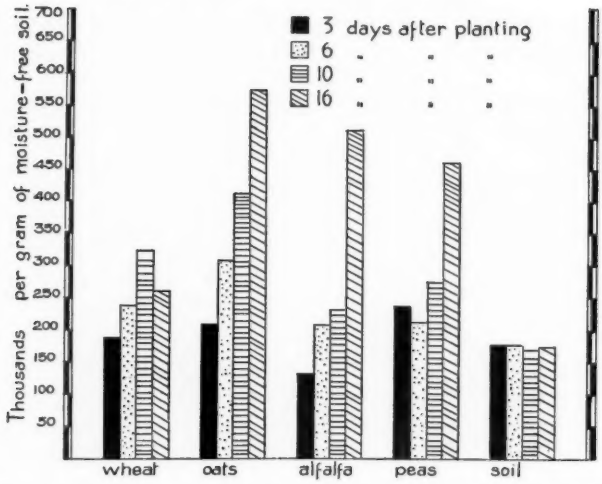

FIG. 2. *Number of fungi in the rhizosphere and soil distant from the roots.*

Qualitative Study of Filamentous Fungi

From Table III it is seen that 16 genera of fungi were recognized in cultures isolated from the rhizosphere and soil distant from the roots. One new species of an emended genus was described.

On examination of the list of genera and species presented in Table III, it is quite evident that there is no significant difference in fungal population in the rhizosphere of the different plants or between the rhizosphere and the soil distant from the roots.

Penicillium, *Trichoderma*, *Fusarium*, and *Coniothyrium* were the prevailing genera in the rhizospheres of all four varieties of plants studied. However, the same genera were also most numerous in the soil distant from the roots. *Phycomycetes* were represented by three genera and were not numerous. *Aspergillus* was represented by only two species, and was not as numerous as *Penicillium*. Species of other genera were obtained so infrequently that they cannot be regarded as characteristic of the different rhizospheres.

An interesting fungus, No. 1121, was isolated from the rhizosphere of alfalfa. It was observed only once on two plates of the same sample, containing respectively two and three colonies of the organism. The conidiophores show a resemblance to the genus *Spicularia* Fuckel. A subculture of this fungus was sent to E. W. Mason, Imperial Mycological Institute, Kew, England. In reply Mr. Mason made the following statement: "The conidiophores and the conidia show a remarkable resemblance to *Spicularia icterus* Fuckel. The original specimen of *Spicularia icterus* is represented at Kew, but I can find no fungus on the leaf. I would rather strongly recommend that the specimen in the herbarium at New York Botanical Garden, Harvard, or Washington be examined."

TABLE III

FREQUENCY OF ISOLATION OF FUNGI IN SOIL NEAR AND DISTANT FROM ROOTS

Fungus	From the rhizosphere of seedlings of				From soil distant from the roots			
	Wheat	Oats	Alfalfa	Peas	First sample	Second sample	Third sample	Fourth sample
Cunninghamella spp.		x		x				
Mucor spp.	x	xx	x	xx		xxx		
Rhizopus nigricans	x	xx	xxx		x			x
Alternaria spp.	x	x					x	
Aspergillus niger	xx						x	
A. versicolor				x		x		
Aspergillus spp.		x			x			
Cephalosporium spp.	x	xx			x			
Cladosporium herbarum	xx	xx	x					
Coniothyrium fuckeli	xx	xx	xxxx	x	x	xx		xxxx
Fusarium spp.	xxxx	xxxx	xxxx	xxx	xxx	xxxx	xxx	xxxx
Gliocladium roseum	xx	xx	x			xx	x	
Mesobotrys spp.			xxx					
Metarrhizium spp.						x		
Monosporium spp.							x	
Penicillium chrysogenum				x		x		
P. intricatum	xx	xx	xx	x		x	xxx	x
P. janthinellum	x	xx		xx	x	x		
P. rugulosum	xx	xxx	xx	x	xx	x	x	xxx
P. tomii		xxx		x			xxx	
P. variabile	x	xx	x		x	x	x	xx
Penicillium spp.	xx	x	xxx	xxx	xxxx	xxx	xx	x
Phoma spp.	x	x			x	x		
Trichoderma album	x							
T. lignorum	xxx		xxxx		xx	x	x	xx
T. koningi		xx	xxx			x		xx
Trichoderma spp.	xxx	xx	x		x		x	x

x = Frequency of organisms isolated from four samples.

The specimen labelled *Spicularia icterus* Fuckel was carefully examined at New York Botanical Gardens by the author, but no fungus was found on the specimen. Dr. H. Linder, curator of the Herbarium of Cryptogamic Botany, Harvard University, and Mr. J. A. Stevenson, Bureau of Plant Industry, Washington, kindly examined the material at their disposal, but the results were also negative. Dr. Linder in his reply stated: "I have just made an examination of No. 2212 of the Fungi Rhenani in a search for *Spicularia icterus* but the only thing found in the meager collection that resembles that specimen is, I am positive, a species of *Botrytis*." Mr. Stevenson also found only a few spherical hyaline conidia approximately 4μ in diameter. From the evidence presented it is quite apparent that there is none of Fuckel's material and no specimen of *Spicularia* Fuckel from any other sources available on this continent or at the Imperial Mycological Institute, Kew, England, with which the fungus No. 1121 could be compared. Therefore the only possible comparison is with Fuckel's description of *S. icterus*.

The generic name *Spicularia* was first established by Persoon (20), but later all his species were transferred by Fries (5) to the genus *Botrytis*. The

genus *Spicularia* Persoon thereupon disappeared until it was brought into being again by Fuckel to take the species *S. icterus* Fuckel. Owing to the fact that spores of *S. icterus* were described as "simplicibus (?)" and "candidis" with hyaline mycelium, Lindau (17) included it in Moniliaceae of the Cephalosporieae subsection with the following criticism, "Fuckel accepts that the fungus causes a disease of grape known by the name yellows. What the author said certainly does not represent the cause of the systematic disease. For example he also introduces the Phyloxera into this discussion. The present day conception of grape yellows indicates that our fungus has no relation to it. It may be traced to soil or climatic conditions. *Spicularia* may then be regarded as a saprophyte upon dry leafspots. The rare occurrence of this fungus apparently substantiates this assumption, because it seems that since Fuckel's time the fungus has never been found again. Fuckel's drawing which has been reproduced herewith does not appear to me to be entirely correct."

Taking into consideration Lindau's criticism of Fuckel's description of *S. icterus* and the fact that there is no material available with which Fuckel's description of *S. icterus* or the fungus No. 1121 could be compared, and since the fungus No. 1121 closely resembles Fuckel's *Spicularia*, we therefore retain the generic name *Spicularia* and the fungus No. 1121 may constitute the type species, unless Fuckel's fungus be collected again and proved to have uniseptate conidia.

Spicularia (Persoon) Em. Nov.

Hyphae creeping, septate, branched, hyaline, or slightly coloured. Conidiophores erect, unbranched, septate, dark olive-brown, bearing at the tip a whorl of club-shaped sterigmata-like cells. Sterigmata are unbranched, non-septate, irregularly swollen at the apex; apex papillate. Conidia are borne singly on each papilla, uniseptate, dark brown, smooth.

Spicularia terrestris n. sp.

Colonies on potato dextrose agar slow spreading, consisting mostly of submerged mycelium and aerial conidiophores; surface velvety in appearance, dark coffee-brown to fuscous; (Ridgway, Plate XLVI); reverse black-brown. In rapidly growing colonies the hyphae near the margin showing a yellow tint. Mycelium branched, sparsely septate with numerous terminal and intercalary dark olive-brown chlamydospores. Conidiophores (Fig. 3) arise from submerged or aerial mycelium, at the point of origin produces 1 to 2 rhizoid-like hyphae; erect, slightly tapering toward apex, unbranched, septate, dark brown up to upper septum, smooth, up to 450μ long. At the apex, which is slightly inflated, bearing a whorl (up to 12) of club-shaped sterigmata-like cells. Sterigmata are unbranched, non-septate and irregularly swollen at the apex covered with numerous papillae. Sterigma are 16 to 18μ long, 3 to 4.5μ wide at the base, and the swollen part up to 8μ thick. Conidia borne singly on each papilla, oblong-ovate, two-celled, slightly constricted at the septum; lower cell often bearing remains of papilla; light brown to fuscous, smooth, 6–9 $\times$ 3–5μ.

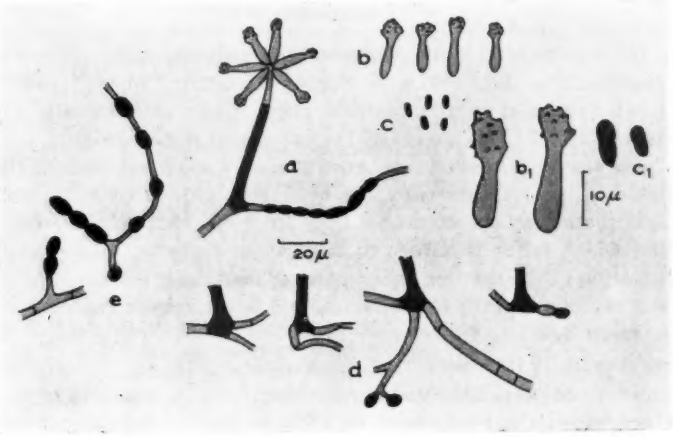

FIG. 3. *Camera lucida drawings of Spicularia terrestris. (a) Conidiophore, (b and b_1) sterigmata, (c and c_1) conidia, (d) bases of conidiophores with rhizoid-like hyphae, (e) submerged hyphae bearing terminal and intercalary chlamydospores.*

Spicularia (Persoon) Em. nov.

Hyphae repentes, septatae, ramosae, hyalinae vel leviter coloratae. Conidiophorae erectae, haud ramosae, septatae, fusco-olivaceae, verticillum cellularum clavatarum similium sterigmatibus apice ferentes. Sterigmata neque ramosa neque septata, apice papillato inaequaliter tumida. Conidia in quaque papilla singulatim gestata, uniseptata, badia, levia.

Spicularia terrestris n. sp.

Societatibus lente extendentibus in agari mixto cum dextro saccharo ex Solano tuberoso exorto, plerumque constantibus ex submerso mycelio et aeriis conidiophoris; superficie visu velutina, caliginosa vel fusca (Ridgway, Plate XLVI) nigrofusca in latere opposito. Hyphis prope marginem societatum crescentium celeriter luteis. Mycelio ramoso, sparsim septato, chlamydosporis plurimis fusco-olivaceis quum terminalibus tum intercalariis. Conidiophoris (Fig. 3) ex submerso aut aerio mycelio exorientibus, loco originis 1–2 hyphas similes radicibus parientibus, erectis, apicem versus leviter tenuius evadentibus, haud ramosis, septatis, caliginosis usque ad septum superum, levibus, usque ad 450μ longis, apice leviter tumido verticillum (usque ad 12 numero) cellularum clavatarum ad similitudinem sterigmatum accedentium gerentibus. Sterigmatibus haud ramosis, haud septatis, apice irregulariter tumido papillis numerosis tecto. Sterigmatibus 16–18μ longis, 3–4.5 μ base latis, parte tumida usque ad 8μ crassa. Conidiis in quaque papilla singulatim partis, oblongo-ovatis, uniseptatis, septo leviter constrictis, fulvis vel fuscis, levibus 6–9 $\times$ 3–5μ, cellula inferiore reliquias papillae saepe ferente.

Discussion

From the results of this investigation it is quite apparent that seedlings, although only three days old, were already harbouring in their rhizospheres from 11 to 28 times as great a bacterial population as elsewhere in the same soil. In the case of fungi, however, the increase is not as striking. The discrepancies of the plate method for the estimation of soil fungi are well known to soil microbiologists. Considering the fact that fungous colonies developing on an agar plate may originate, not only from spores, but also from bits of mycelium, which latter is harder to disperse or to break during shaking in the preparation of the dilution than spores or conidia, it is quite possible that increase of mycelium in the rhizosphere could be far greater than indicated by the plate method.

A second point of interest is that the duration of the experiment (15 days) is not sufficient to permit the assumption that the seedlings deposited enough organic residue of the root tissue to account for the increase of microbial activity noted. While it is true enough that there is some sloughing of epithelial cells from the growing root tips which may favour microbial development to some extent, it is considered that the excretion of water-soluble chemical compounds by the roots is the chief cause of the increased microbial activity in the rhizosphere. To elucidate these questions further research is necessary.

Acknowledgments

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PRUNUS IN EASTERN CANADA[1]

By Herbert Groh[2] and Harold A. Senn[2]

Abstract

The distribution of *Prunus* species in Eastern Canada is of definite economic significance since these species serve as secondary hosts for leaf hoppers and aphids, which are vectors of important virus diseases.

A key is presented for the determination of the following native and naturalized species: *Prunus spinosa, insititia, domestica, americana, nigra, Persica, pumila, avium, Cerasus, mahaleb, pennsylvanica, serotina, Padus,* and *virginiana.* The detailed distribution of these species is outlined through the citation of herbarium specimens and Canadian Weed Survey records, the ranges of the more important species being mapped.

Cultivated and naturalized species are considered to be of little significance in disease transmittal. In potato-growing regions the choke cherry (*P. virginiana*) which frequents fence-rows is probably the most important species, although the bird cherry (*P. pennsylvanica*) may also be important on light sandy soils.

Introduction

Recent enquiries from entomologists and plant pathologists regarding the distribution and incidence in Eastern Canada of certain wild plums and cherries were the immediate occasion for the preparation of this paper.

It has been represented that plums occurring in the Niagara fruit belt are the hosts of the leafhopper vector of peach virus diseases. Investigations proceeding in the potato-growing districts of Eastern Canada also indicate that *Prunus* species serve as the overwintering hosts of the species of aphid most concerned in the distribution of potato diseases.

Trial tabulations of some of the *Prunus* records available in the Canadian Weed Survey files in the Division of Botany and Plant Pathology were made by Miss Jean C. Burnham, Dominion Entomological Laboratory, Fredericton, N.B. These, together with the subsequent studies here reported, are a definite indication of the value of having an adequate knowledge of the distribution of Canadian plants generally, upon which knowledge many details of agricultural practice have to be based. In view of what had been disclosed the Dominion Botanist urged that all the botanical information available should be properly compiled and placed at the disposal of all workers interested.

The records referred to above were quite fortuitously available for the use suggested, having been secured in the course of weed surveys initiated in 1923 and still continuing. Partly as fence-row or waste land encumbrances, but chiefly because of reputed responsibility for prussic acid poisoning of animals browsing upon the wilted foliage of *Prunus* species, the two most generally common members of the genus, *P. virginiana* L., the choke cherry,

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and *P. pennsylvanica* L.f., bird or wild red cherry, had been admitted to the list of weeds under survey. For other than those two species no such source of information was at hand. Specimens had not ordinarily been collected of either these or other species. A certain amount of information was available in the literature, but it was soon realized that this had to be accepted with a good deal of discrimination, particularly in the case of some species. Taking all things into account it was deemed advisable to assemble and make the widest possible examination of herbarium material. For this purpose the larger and most of the smaller collections in Eastern Canadian and United States institutions were placed freely at our service. Of most species enough material was thus available to give a broad outline, at least, of the desired range limitations within the eastern half of Canada.

In one respect the extensive citations that follow are not all that is to be desired. A rather large proportion of those of most native species are from areas outside of present or likely agricultural settlement, owing to an apparent flair of botanists for collecting in more virgin fields. For the study of incidence here required they tend to misplace emphasis. This is in some contrast with the weed survey records already referred to which were secured largely from within the territory to be served by such a study as this. The two types of data are required to complement each other, even if not equivalent in all respects.

The objection which will be raised in some quarters that sight records, unsupported by specimens, are invalid evidence should not be given undue weight in the case of two species as readily distinguishable in the field as the choke cherry and the bird cherry of Eastern North America. The occasional occurrence of black cherry in the more southerly part of their range can be a source of confusion at times. Beyond our westerly limits, also, eastern and western species of slightly differing appearance would be a problem. Granted that misidentifications in the field can occur and cannot be checked afterwards, it may still be affirmed that the wealth of records here personally secured by careful reconnaissance methods is amply large to minimize any error which may chance to be included. If obviously extra-territorial, a record unsupported by a specimen would be rejected; within the expected range, the most that a chance error can do to the general result is to alter by one in the total the frequency index secured.

The present treatment includes all the native *Prunus* species of Eastern Canada and all the species that have become established as escapes from cultivation. It does not include the species that are cultivated primarily as ornamentals, since no records were discovered to indicate that such species had become naturalized. This should not, however, be interpreted as an indication that such species may not play a part in the overwintering of the insects mentioned above.

Since no key was available for the determination of the group of species within the purview of this study the key presented below was prepared. The more important North American synonymy for each species has been included,

but no attempt has been made to present a complete bibliography, especially of Asiatic and European species.

Methods

Eastern Canada is here taken to mean west to Meridian 92°, which divides the settled belt more equally into halves for mapping than would be the case if the Manitoba-Ontario boundary were used. Exigencies of mapping are also allowed to exclude Newfoundland on the east, for which there are citations in some instances but not survey records.

The ranges of the native species for which no weed survey records were available (*Prunus americana*, *nigra*, *pumila*, and *serotina*) have been mapped from cited specimens on outline maps (see Figs. 1, 2, 3, 4, 5, 8).

Special maps have been prepared for *P. pennsylvanica* (Fig. 7) and *P. virginiana* (Fig. 9) including both citations of herbarium specimens and Weed Survey records. Fig. 6 records all points at which weed surveys were made, thus indicating by comparison with Figs. 7 and 9 the points at which surveys were made but no *Prunus* was found.

Instead of a standard base map an outline map has been fitted to squared paper in an approximation to a Mercator's projection. Latitude and longitude are thus conveniently used, instead of confusing map detail, to locate position. When surveys are repeated at any location, displacement of entry on the map is kept as small as possible and within the correct meridian. Entry of herbarium records is also kept more strictly to meridians than to parallels where crowding occurs.

Material Examined

Specimens were seen from the herbaria designated as follows:

A	Acadia University, Wolfville, N.S.
AA	Arnold Arboretum of Harvard University, Jamaica Plain, Mass.
Can	National Herbarium of Canada, Ottawa, Ont.
Dal	Dalhousie University, Halifax, N.S.
DAO	Division of Botany and Plant Pathology, Science Service, Dominion Department of Agriculture, Ottawa, Ont.
G	Gray Herbarium of Harvard University, Cambridge, Mass.
M	University of Montreal, Montreal, Que.
McG	McGill University, Montreal, Que.
McM	McMaster University, Hamilton, Ont.
MV	Private herbarium of Prof. Marie-Victorin, University of Montreal, Montreal, Que.
NBM	New Brunswick Museum, Saint John, N.B.
OAC	Ontario Agricultural College, Guelph, Ont.
Q	Queen's University, Kingston, Ont.
SteA	Ecole Supérieure d'Agriculture, Ste. Anne de la Pocatière, Que.
T	University of Toronto, Toronto, Ont.
US	United States National Herbarium, Washington, D.C.

Acknowledgments

To the custodians of these various collections our sincere thanks are extended for the courtesies shown. To others, whether named in these pages or not, who have given information or have helped by interest taken in the project, and to Messrs. W. H. Minshall and M. N. Zinck of this Division, for valued assistance in the preparation of the maps, we are also grateful.

Key to the Native and Naturalized *Prunus* Species of Eastern Canada

A. Leaves conduplicate or convolute in the bud; flowers opening before or at the same time as the appearance of the leaves; drupe sulcate, pubescent or with a bloom. Plums and Peaches.....B.

B. Axillary buds solitary, terminal bud wanting; flowers pedicelled in simple umbel-like clusters, white or greenish-white (very rarely pink); drupe glabrous with a bloom; stone smooth or slightly sculptured. Plums.....C.

C. Leaves convolute in the bud; flowers 1–2 per cluster; stone somewhat sculptured. European and Asiatic species escaped from cultivation in Canada.....D.

D. Low shrub, distinctly spiny; young branchlets pubescent; leaves small, 2–4 cm. long; flowers usually solitary; pedicels glabrous, rarely pubescent; fruit globose, small, usually less than 1 cm. in diameter.....1. *P. spinosa*

D. Shrub or small tree, unarmed or slightly spiny; young branchlets glabrous or pubescent; leaves larger, 4–10 cm. long; flowers 1–2; pedicels pubescent, rarely glabrous; fruit globose or variable in shape, larger, more than 1 cm. in diameter.....E.

E. Shrub or small tree, sometimes slightly spiny, of dwarf compact habit; young branchlets pubescent; leaves relatively small, 4–8 cm. long, tending to be more ovate, with closely serrate margins; pedicels pubescent; fruit regular, globose or ovoid, usually less than 2–5 cm. in diameter.....2. *P. insititia*

E. Small tree, unarmed, of erect habit; young branchlets glabrous or rarely slightly pubescent; leaves relatively large, 5–10 cm. long, tending to be more elliptic or obovate, with coarsely irregularly serrate margin; pedicels glabrous, rarely pubescent; fruit variable in shape, large, usually more than 2.5 cm. in diameter.....3. *P. domestica*

C. Leaves conduplicate in the bud; flowers usually 3 or more per cluster; stone usually smooth. American species.....F.

F. Leaf serrations pointed, acute, bristle tipped; calyx lobes entire, devoid of glands; inner surface of the calyx lobes densely pubescent.....4. *P. americana*

F. Leaf serrations rounded, obtuse, glandular; calyx lobes serrate, glandular; inner surface of the calyx lobes glabrous or sparsely pubescent ..... 5. *P. nigra*

B. Axillary buds 3; terminal bud present; flowers subsessile, usually solitary, pink; drupe pubescent (very rarely glabrous); stone deeply sculptured. Peaches ..... 6. *P. Persica*

A. Leaves conduplicate in the bud; flowers usually opening after the appearance of the leaves; drupe not sulcate, neither with a bloom nor pubescent. Cherries ..... G.

G. Flowers solitary or few, in umbels or in short, few-flowered racemes; bracts, conspicuous ..... H.

H. Shrubs; buds 3 in each leaf axil; pedicels usually short. Sand cherry ..... 7. *P. pumila*

H. Trees or tall shrubs; buds solitary; pedicels usually long ..... I.

I. Leaves dentate with obtuse teeth; involucral bracts at base of the inflorescence persistent; flowers in umbels. Cultivated Eurasian cherries, escaped in Canada ..... J.

J. Trees, not suckering from the root; leaves long (up to 15 cm. long), pubescent beneath, at least on the veins; inner bud scales reflexed; inflorescences without leafy bracts; calyx lobes entire; fruit sweet ..... 8. *P. avium*

J. Trees, suckering from the root; leaves short (up to 7 cm. long), glabrous; inner bud scales erect; inflorescence usually with a few leafy bracts; calyx lobes crenate; fruit sour ..... 9. *P. Cerasus*

I. Leaves crenate-serrulate with sharp pointed teeth; involucral bracts at the base of the inflorescence deciduous; flowers in umbels or short racemes ..... K.

K. Leaves sub-orbicular or broad-ovate, crenulate-serrate; flowers in short racemes ..... 10. *P. mahaleb*

K. Leaves oblong-lanceolate, acuminate or rarely acute at the apex, finely and sharply serrate with incurved teeth; flowers in umbels or corymbs ..... 11. *P. pennsylvanica*

G. Flowers in elongated racemes with numerous flowers; bracts small ... L.

L. Leaves serrulate with incurved callous teeth; calyx persistent in fruit ..... 12. *P. serotina*

L. Leaves sharply serrate with slender teeth; calyx deciduous in fruit ..... M.

M. Leaves elliptic to obovate or oblong-ovate, rounded or subcordate at the base, sharply serrate; calyx-tube pubescent inside; petals twice as long as the stamens; stone sculptured ..... 13. *P. Padus*

M. Leaves broad-elliptic to obovate, broad-cuneate to rounded at the base, closely serrulate; calyx-tube glabrous inside; petals less than twice as long as the stamens; stone smooth ..... 14. *P. virginiana*

SUBGENUS I. *Prunophora* Focke

Section 1. *Euprunus* Koehne

1. *Prunus spinosa* L.

Sp. Pl. 475. 1753; Torr. & Gray, Fl. N. Amer. 1: 408. 1840; Bailey, Cycl. Am. Hort. 1447. fig. 1979. 1901; Hedrick, Plums of N.Y. 42. 1911 (see this for synonymy); Rehder, Man. Cult. Trees and Shrubs, 454. 1927.

Common name: Blackthorn (Sloe).

Distribution: Europe, North Africa, Western Asia; introduced and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Summerville, *Jack 3490* (G), escaped along roadside; Wolfville, *Cameron*, May 6, 1936 (A).

ONTARIO: Galt, *Herriott*, May 30, 1909 (G, Can); Pelee Island, *Macoun*, July 27, 1892 (Can).

2. *Prunus insititia* L.

Cent. Pl. 1: 12. 1755; Amoen. Acad. 4: 273. 1759, non Walt. Fl. Carol. 146. 1788; Robins. & Fern. in Gray's Man. ed. 7, 498. 1908; Hedrick, Plums of N.Y. 34. 1911 (see this for synonymy); Britt. & Brown, Illus. Fl. ed. 2, 2: 326. 1913; Rehder, Man. Cult. Trees and Shrubs, 454. 1927.

Prunus spinosa insititia (L.) A. Gray, Man. ed. 2, 112. 1856.

Common name: Bullace (Damson).

Distribution: Western Asia, Europe; introduced and escaped in Eastern Canada.

Specimens examined:

QUEBEC: Ste. Anne de la Pocatière, *Campagna 8036* (SteA), dry sand; Berthier-en-bas, *Marie-Victorin, Rolland-Germain & Jacques 33194* (AA, MV, M).

3. *Prunus domestica* L.

Sp. Pl. 475. 1753; Bailey, Cycl. Am. Hort. 1447. 1901, in part; Hedrick, Plums of N.Y. 12. 1911 (see this for synonymy); Rehder, Man. Cult. Trees and Shrubs, 455. 1927; Marie-Victorin, Fl. Laurent. 320. 1935.

Common name: Garden plum.

Distribution: Western Asia and Europe; cultivated and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Halifax, *Jack 3238, 3649* (AA), naturalized; Pictou, *Jack 784* (AA) naturalized, common.

Section 2. *Prunocerasus* Koehne

4. *Prunus americana* Marshall,

Arb. Am. 111. 1785; Torr. & Gray, Fl. N. Amer. 1 : 407. 1840, in part; Torr. Fl. N.Y. 1: 194. 1843, in part; Sargent, Silva N. Amer. 4: 19. pl. 150. 1892 (see this for full synonymy); Bailey, Cycl. Am. Hort. 1448. 1901; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Hedrick, Plums of N.Y. 56. 1911; Britt. & Brown, Ill. Fl. ed. 2, 2: 323. 1913; Wight, Nat. Am. Sp. Prunus 24. 1915 (U.S. Dept. Agr. Bull. 179); Rehder, Man. Cult. Trees and Shrubs, 458. 1927.

Prunus ignotus Nelson, Bot. Gaz. 42: 53. 1906.

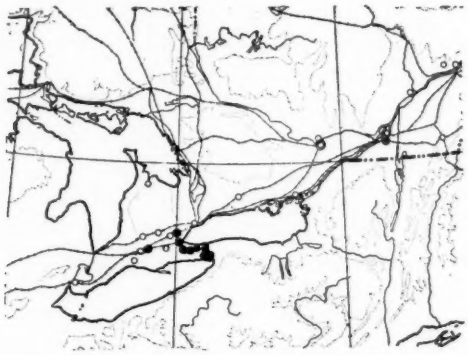


FIG. 1. *Range of Prunus americana Marsh., Wild plum.*

Common name: Wild plum.

Distribution: Eastern and Central United States and southern Ontario; extending into Canada again in southern Manitoba. See Fig. 1.

Specimens examined:

QUEBEC: Caughnawaga, *Jack*, July 30, 1900, Aug. 26, 1908 (AA), probably escaped from plantings.

ONTARIO: Guelph, *Simmons* May 30, 1935 (OAC), hedgerows, *in litt.* reported as escape; (?Hamilton), *Dickson*, May 2, 1895 (McM); Lincoln Co., *McCalla 567* (US); Queenston, *Scott*, Aug. 6, 1902 (Can), *Putman & Armstrong*, May, 1938 (DAO), near Niagara, *Macoun*, May 11, 1901 (Can); Niagara River between Queenston and Niagara-on-the-Lake, *Putman & Armstrong*, May, 1938 (DAO); Chautauqua Park, Niagara-on-the-Lake, *Putman & Armstrong*, May, 1938 (DAO); Jordan Station, *Putman & Armstrong*, May, 1938 (DAO); St. David's, *Putman & Armstrong*, May, 1938 (DAO); Rockway, *Putman & Armstrong*, May, 1938 (DAO); Power Glen, De Cew Falls, *Putman & Armstrong*, May, 1938 (DAO); London, *Burgess*, May 24, 1885 (McG), *Balkwill*, Sept. 9, 1885 (McG).

Mr. W. R. Leslie, Superintendent, Experimental Station, Morden, Man., who has studied the wild plums in Manitoba, in a letter dated December 16, 1938, expresses the view that *P. americana* occurs in the limestone areas, and

P. nigra in the granitic areas to the east and north, although they intermingle along the Red River, especially at Winnipeg. In southern Ontario *P. americana* ranges far short of limestone limits.

5. *Prunus nigra* Ait.

Hort. Kew. 2: 165. 1789; Sargent, Silva N. Amer. 4: 15. pl. 149. 1892 (see this for full synonymy); Small, Bull. Torr. Bot. Club, 21: 301. 1894; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Hedrick, Plums of N.Y. 69. 1911; Britt. & Brown, Ill. Fl. ed. 2, 2: 323. 1913; Wight, Nat. Am. Sp. Prunus, 21. 1915 (U.S. Dept. Agr. Bull. 179); Sargent, Man. Trees N. Amer. ed. 2, 560. 1922; Rehder, Man. Cult. Trees and Shrubs, 458. 1927; Marie-Victorin, Fl. Laurent. 320, 1935.

Prunus americana var. *nigra* Waugh, Vt. Agr. Exp. Sta. Bull. 53: 60. 1896; Vt. Agr. Exp. Sta. Ann. Rep. 10: 102. 1897; Bailey, Cycl. Am. Hort. 1449. 1901.

Prunus americana American authors, in part.

Common name: Canada plum.

Distribution: Central and northeastern Canada and United States; as far west as the Riding Mts. in Manitoba. See Fig. 2.

Specimens examined:

NOVA SCOTIA: Lansdowne, *Jack 3705* (AA, G), roadside thicket; Wolfville, *Perry*, May 21, 1912 (A); Gaspereau Valley, *McLellan*, June 1, 1939 (DAO).

NEW BRUNSWICK: St. John, *Hay*, June, 1897 (G).

QUEBEC: Quebec, Plains of Abraham, *Michel 1638* (M); Ancienne Lorette, *Marie-Victorin 15535* (M, MV); Lake Memphremagog, Georgeville to Fitch Bay, *Churchill*, Aug. 16, 1903 (G), roadside; Lake Memphremagog, near Oliver Corner, *Churchill*, Aug. 22, 1919 (G), roadside; Pointe du Lac, *Stanislas 444* (M); St. Laurent, *Adrien 20911* (M) *1080* (M, MV); Ahuntsic, *Masson*, May 16, 1925 (M); Ste. Julie de Vercheres, *Marie-Victorin 28636* (M); Cartierville, *Adrien*, May 22, 1925 (M, MV); Ile Bizard, *Adrien, 1714* (M, MV); Montreal, *Robert 22* (M); Longueuil, *Marie-Victorin 189, 4330, 9508, 2093* (MV), *3400* (US), *Marie-Victorin & Rolland-Germain 45203* (M, G), *Rolland-Germain 29420, 29465* (M, MV); Mount Royal, Montreal, *Marie-Victorin 34180* (M, MV); Caughnawaga, *Jack*, May 20, 1900 (AA), *Woodson & Anderson*, Sept. 18, 1931 (G), woodlands, hedgerows; Chateauguay, *Jack 3966* (US); Vaudreuil, *Jack*, Sept. 23, 1913 (AA); Hull, *Harrington*, May 19, 1906, May 23, 1908 (Can); Fairy Lake (near Hull), *E. G. Anderson*, no date (OAC); Farm Point, *Macoun 87170* (Can).

ONTARIO: Summerstown, *Jack*, May 24, 1914 (AA); Cornwall, *Jack*, May 28, 1913, Sept. 24, 1913 (AA); 2 mi. east of Iroquois, *Minshall 1215* (DAO); Ottawa, *Ami*, May, 1879 (McG), *Fletcher*, April 30, 1878, dry woods at Billings Bridge, May 24, 1906 (DAO), *Scott*, May 24, 1890 (OAC), *Macoun*, Aug. 4, 1894 (Can), Billings Bridge, *J. M. Macoun*, May 14, 1891 (Can), Beechwood, *Groh*, April 30, 1910 (DAO), *Minshall*, May 15, 1934 (DAO), Dow's Swamp; Carlsbad Springs, *Macoun*, June 6, 1911 (Can); Stittsville, *Macoun*, May 8, 1903, (Can); Gananoque, *Fowler*, Aug. 18,

1887 (Q); Kingston, *Fowler*, May 23, 1884, May 16, 1900 (Q), May 29, 1905, May 12, 1896, May 7, 1894, May 11, 1901 (US), *Millman*, Sept. 9, 1885 (T, McG); Belleville, *Macoun*, May 24, 1878 (T, Can); Hastings Co., *Macoun*, May 4, 1870 (McG); Toronto, *Scott*, May 14, 1898 (Can), in Don Valley; Arkell, *Bot. Dept., O.A.C.*, May 14, 1931 (OAC); Preston, *Groh*, Dec. 30, 1938 (DAO); Toronto, Bathurst St., near Eglinton, *R. W. Smith*, June 2 (McM); Queenston, *Scott*, Aug. 6, 1902 (T); Niagara Falls, *Macoun*, May 13, 1901 (Can); London, *Millman*, April 30, 1879 (T), *Burgess*, June 21, 1879 (McG); Aylmer, *R. T. Anderson*, May 8, 1898 (T), woods and river banks; Opeongo Lake, Algonquin Park, *Macoun*, Aug. 17, 1900 (T); 51-mile post, District of Thunder Bay, *Macinnis*, Aug. 1900 (Can).

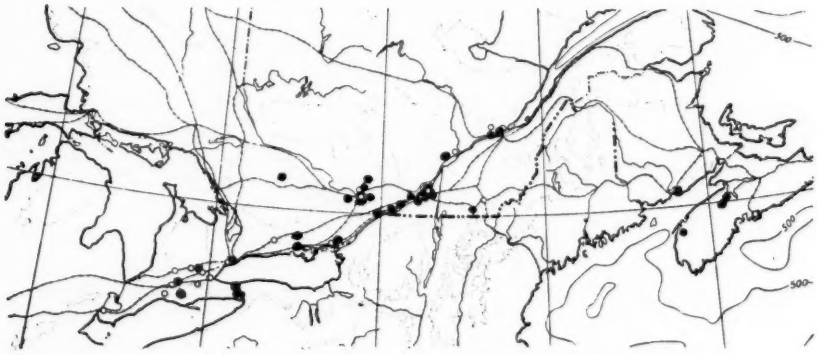


FIG. 2. *Range of Prunus nigra Ait., Canada plum.*

SUBGENUS II. *Amygdalus* Focke

6. *Prunus Persica* (L.) Batsch.

Beytr. Entw. (Pragm.) Gesch. Naturw. 30. 1801*; Bailey, Cycl. Am. Hort. 1457. 1901; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Hedrick, Peaches of N.Y. 77. 1917 (which see for full synonymy); Rehder, Man. Cult. Trees and Shrubs, 462. 1927.

Amygdalus Persica L. Sp. Pl. 472. 1753; Britt. & Brown, Illus. Fl. ed. 2, 2: 330. 1913.

Persica vulgaris Mill. Gard. Dict. ed. 8, No. 1. 1768.

Common name: Peach.

Distribution: China, cultivated and locally escaped in southern Ontario.

Specimens examined:

ONTARIO: Queenston, *Scott*, May 18, 1901 (T); Lake Erie, Point Pelee National Park, *Senn 1044* (DAO), escaped along sandy beach.

This is to be expected as an escape in the peach belt, and is not unknown beyond it. A small tree growing on the southern border of woods near Preston, Ontario, about 1915, was found bearing fruit one year.

* *See Junger in Botan. Z. 36: 426. 1878, Koehne in Mitt. Deutsch. Dendr. Ges. 26: 69. 1917.*

SUBGENUS III. *Cerasus* Pers.

Section I. *Microcerasus* Webb

7. *Prunus pumila* L.

Mant. 75. 1767; Bailey, Cycl. Am. Hort. 1450. 1901; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Britt. & Brown, Ill. Fl. ed. 2, 2: 326. 1913; Wight, Nat. Am. Sp. Prunus 65. 1915 (U.S. Dept. Agr. Bull. 179); Hedrick, Cherries of N.Y. 34. 1915 (in part); Fernald in Rhodora, 25: 72. 1923; Rehder, Man. Cult. Trees and Shrubs, 466. 1927.

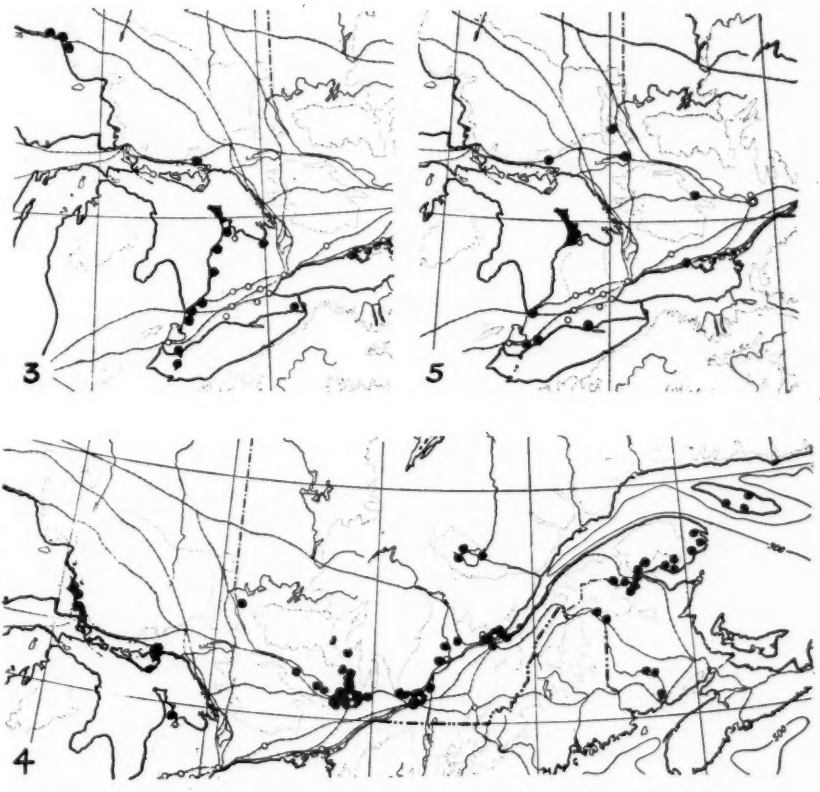
Cerasus canadensis Mill. Gard. Dict. ed. 8. 1768.

Cerasus glauca Moench, Meth. 672. 1794.

Cerasus pumila (L.) Michx. Fl. Bor.-Am. 1: 286. 1803 as to name-bringing synonym only.

Common name: Sand cherry.

Distribution: Eastern Canada and northeastern United States. See Figs. 3, 4, and 5.



FIGS. 3, 4, 5. *Range of Prunus pumila L., Sand cherry.* 3. *P. pumila L. var. typica.* 4. *P. pumila L. var. depressa (Pursh) Bean.* 5. *P. pumila L. var. susquehanae (Willd.) Jaeg.*

We are unable to follow Fernald (Rhodora, 25: 69. 1923) in recognizing as distinct species the three sand cherries of Eastern Canada which have been known as *Prunus pumila* L., *P. depressa* Pursh, and *P. susquehanae* Willd. (*P. cuneata* Raf.). These entities show a complex series of intergradations in the morphological characters which Fernald uses to separate them. In habit the first and last are essentially erect except that *P. pumila* in age tends to become decumbent at the base. *P. depressa* is as a rule, low and spreading. There is considerable intergradation in leaf shape. The leaves of the first two entities are characteristically more elliptical with acute or acuminate apices while the leaves of the latter are more obovate and obtuse. However the lower leaves of *P. depressa* are frequently obovate obtuse and almost indistinguishable from *P. susquehanae*. Wight (Nat. Amer. Sp. *Prunus*, 67. 1915, U.S. Dept. Agr. Bull. 179) while regarding *P. pumila* (including *P. depressa*) and *P. susquehanae* (*P. cuneata*) distinct as regards habit, says "Even mature foliage (of *P. susquehanae*) sometimes approaches *P. pumila* so closely as to render separation difficult, though they are usually much broader toward the base than in the former species."

Fernald (l.c.) placed considerable emphasis on the edibility of the fruit of *P. depressa* in contrast to the small unsatisfactory fruits of *P. pumila*. Yet Bailey (Nat. Dwarf Cherries, Cornell Univ. Agr. Exp. Sta. Bull. 70. 260. 1894) writes of the Great Lakes plant, *P. pumila*:

"This sand cherry is variable in its wild state, especially in its fruit. As a rule, the fruit is small and very sour and scarcely edible, but now and then one comes upon a bush which has fruit of pleasant flavour and as large as small Early Richmond cherries."

The three entities have a somewhat distinct distribution (see Figs. 3, 4, 5), *P. pumila* being centered about the Great Lakes, *P. depressa*, being eastern and northern, and *P. susquehanae* ranging from Pennsylvania across New England and north to Lake Superior. Additional collections seem to be rapidly destroying any supposedly definite boundaries between these forms.

Consequently since there is considerable intergradation in morphological characters and not too sharply delimited geographical ranges we consider that these sand cherries should be regarded as varieties of a polymorphous species rather than as distinct species.

Key to the Varieties of *P. pumila*

A. Shrub, erect or prostrate; leaves usually elliptic to oblanceolate-spatulate, usually acute, relatively thin.

B. Shrub erect (reaching 1.5–2 m. in height) in age decumbent at the base; leaves elliptic, narrow, rarely tending to be oblanceolate-spatulate, acuminate or acute, cuneate at the base, with close sharp divergent serrations; fruit usually small, scarcely edible. Great Lakes.....1. var. *typica*

B. Shrub prostrate (rarely over 0.3 m. in height); leaves broader, oblanceolate-spatulate, usually acuminate or acute, but the lower leaves occasionally obtuse, serrations tending to be more crenate; fruit larger, edible. Eastern and northern regions.....2. var. *depressa*

A. Shrub, erect; leaves obovate-spatulate, usually short, obtuse, thicker. Ontario.....3. var. *susquehanae*

Prunus pumila L. var. *typica*

Specimens examined:

ONTARIO: Wellington, *Fowler*, Sept. 3, 1902 (G); Point Abino, Lake Erie, *Coville*, Aug. 23, 1886 (US), *Keller*, July 11, 1925 (US); Pt. Pelee, *Macoun*, June 29, 1882 (US, Can), *Burgess*, June 30, 1882 (DAO, T), July 1, 1882 (McG); Pelee Island, *Macoun*, July 27, 1892 (Can); Point Edward, *Macoun*, June 15, 1901, (G, Can); Forest, *Howitt*, June 11, 1914 (OAC); near Sarnia, *Dodge*, May 2, June 19, 1893 (Can); sandy places near Pt. Frank, *Baker* (OAC); Wasaga Beach, Simcoe Co., *Marie-Victorin, Rolland-Germain & Meilleur 45106* (M, T); Johnston Harbour, Lake Huron, *Macoun*, Aug. 24, 1901 (G); Stokes Bay, *Krotkov 9148* (T); Big Bay, Tobermory, *Krotkov 7533* (T,G) *7534* (T); Oliphant, Lake Huron, *Breed, Jeffrey, Stebbins, et al. 166* (G); Georgian Bay Biological Station, no data (Can); Cloche Bluff, McGregor Bay, north side of Georgian Bay, *Coville*, July 25, 1915 (US); Lake Superior, *Loring*, no date (G); north shore of Lake Superior, *Macoun*, 1869 (US); Peninsular Harbour, north shore of Lake Superior, *Miller*, Sept. 26, 1896 (G).

7a. *Prunus pumila* L. var. *depressa* (Pursh) Bean,

Trees and Shrubs Hardy in the Br. Isles 2: 249. 1914; Rehder, Man. Cult. Trees and Shrubs 466. 1927.

Prunus depressa Pursh. Fl. Am. Sept. 1: 332. 1814; Fernald in Rhodora, 25: 73. 1923; Marie-Victorin, Fl. Laurent. 320. 1935.

Cerasus pumila Michx. Fl. Bor. Am. 1: 286. 1803, as to shrub described.

Prunus pumila Torr. Fl. N.Y. 1: 195. 1843, non Linn.

Specimens examined:

NEW BRUNSWICK: Drury's Cove, *Hay*, June 15, 1878 (NBM); Grand Lake, *Hay*, July 1, 1876 (Can, Q), another specimen of this collection under the name *pumila*, in Acadia University Herbarium, was not seen by us; Goat Island, Grand Lake, *Fowler*, Sept. 17, 1879 (Q); shores of Upper Restigouche, *Hay*, July 28, 1896 (NBM); Tom's Island, Restigouche River, *Hay*, July 30, 1896 (G); St. John River between Edmundston and Grand Falls, *Hay*, July 19, 1879 (NBM).

QUEBEC: Rivière au Saumon, *Marie-Victorin, Rolland-Germain & Louis-Marie 20888* (MV); Rivière de Brig, Anticosti, *Macoun*, Aug. 10, 1883 (Can); Rivière McKane, Anticosti Id., *Marie-Victorin & Rolland-Germain 27366* (M, MV); Ile à Deux Têtes, L'Estuaire des Saint Laurent, *Rousseau 24658* (US, Can, M, MV); Grand River, Gaspé Co., *Richards*, June 20–July 10, 1903 (G); Mt. St. Pierre, Gaspé Co., *Pease & Goodale*, July 13,

1932 (G), *Fernald, Weatherby & Stebbins 2453* (G, Can, M); Gaspé, *Macoun*, 1882 (Can); Rivière Bonaventure, *Marie-Victorin, Rolland-Germain & Jacques 33263* (M, MV), *45053* (M, Can); Bonaventure River between Baldé and Baie de Chaleur, *Collins, Fernald & Pease, 5829, 5830* (G); Rivière Bonaventure, *Campagna, Arsenault & Bauville*, Aug. 12, 1931 (SteA); Matapedia, *Fernald*, June 28, 1904 (G), *Marie-Victorin 28683* (M, MV); Milnekek, Matapedia River, *Rousseau & Bonin 32039* (M, MV), *32083* (M, US, Can); Restigouche River, 20 mi. below Patapedia River, *Rousseau & Bonin 32218* (Can, M); Junction Restigouche and Patapedia Rivers, *Rousseau & Bonin 32155* (M, MV, Can, US); Lake St. John, *Jack*, Aug. 23, 1895 (AA); Grand Discharge, Lake St. John, *Kennedy*, July 22, 1892 (G), *Marie-Victorin, Rolland-Germain & Meilleur 43031* (M), *LV*, Aug. 1921 (M); Pointe Bleue, Lac St. Jean, *Marie-Victorin 15539* (US, M, MV); Pointe Sud-Ouest de l'Ile d'Orleans, *Marie-Victorin 15538* (AA, US, M, MV); Ste. Petronille, Ile d'Orleans, *Marie-Victorin, Rolland-Germain & Meilleur 45026* (M); St.-Lambert de Laizon, Levis Co., *Marie-Victorin, Rolland-Germain & Meilleur 30535* (M); Pont Rouge, *Marie-Anselme*, Aug. 21, 1938 (DAO); St. Georges, Beauce Co., *Marie-Victorin, Rolland-Germain & Meilleur 43342* (M); Pointe-au-Lac, St. Maurice Co., *Stanislas 445, 446* (M); Shawinigan Falls on St. Maurice River, *Chamberlain & Knowlton*, Aug. 1, 1923 (G); St. Genevieve, Anse à l'Orme, *Adrien*, July 29, 1927 (M, MV); Contrecoeur, Vercheres Co., *Marie-Victorin & Rolland-Germain 29212* (M, MV); Portage de Gres River, Montreal, *Holmes*, July 10, 1882 (McG); between Longueuil and Isle Ste.-Helene, *Marie-Victorin 8340* (MV); Ste. Rose, Laval Co., *Marie-Victorin & Rolland-Germain 43741* (M); Oka, Lake of Two Mountains, collector not stated, 1909, *Marie-Victorin*, July, 1914 (MV); vicinity of Ottawa, *Marie-Victorin, 9130* (M), *10114* (M, MV); Ottawa River, *Rolland 55* (M, MV); vicinity of Ottawa, *Rolland, 7159* (MV); Brigham's Creek, Hull, *Macoun*, Sept. 11, 1894 (Can); island at Chelsea, *Macoun*, July 27, 1911 (Can), approaching *susquehanae;* Kirk's Ferry, *Harrington*, May 20, 1906, July 5, 1908 (Can); Paugan Falls, *Macoun*, Sept. 3, 1894 (Can); Ile des Allumettes, *Marie-Victorin, Rolland-Germain & Meilleur 44946* (M); Kazabazua, *Dore*, June 2, 1932 (DAO); Norway Bay, *Adams*, July 23, 1917 (DAO); Pointe au Vent, Lake Temiscaming, *Marie-Victorin 8285* (AA, MV).

ONTARIO: Ottawa, Rockcliffe Park, *Macoun*, May 26, 1903, (Can, DAO), May 18, 1911 (Can), Chaudière Islands, *Fletcher*, June 3, 1883 (McG, DAO), *Scott*, June 6, 1890 (OAC), May 24, 1891, June 6, 1892 (T), Beechwood, *Scott*, May 24, 1893, May 24, 1898 (T), near Lemieux Island, *Minshall*, May 27, 1937 (DAO); Constance Bay, *Groh*, May 13, 1930, *Minshall*, May 17, 1934, *Adams*, June 12, 1938 (DAO); Chalk River, *Macoun*, June 30, 1884 (Can, McG); Howdenvale, Bruce Co., *Watson 2942* (US, T); Great Cloche Island, Manitoulin District, *Fernald & Pease* 3398 (G, T); Pancake Bay, *Taylor, Losee & Fitzpatrick 2115* (T); Batchewana

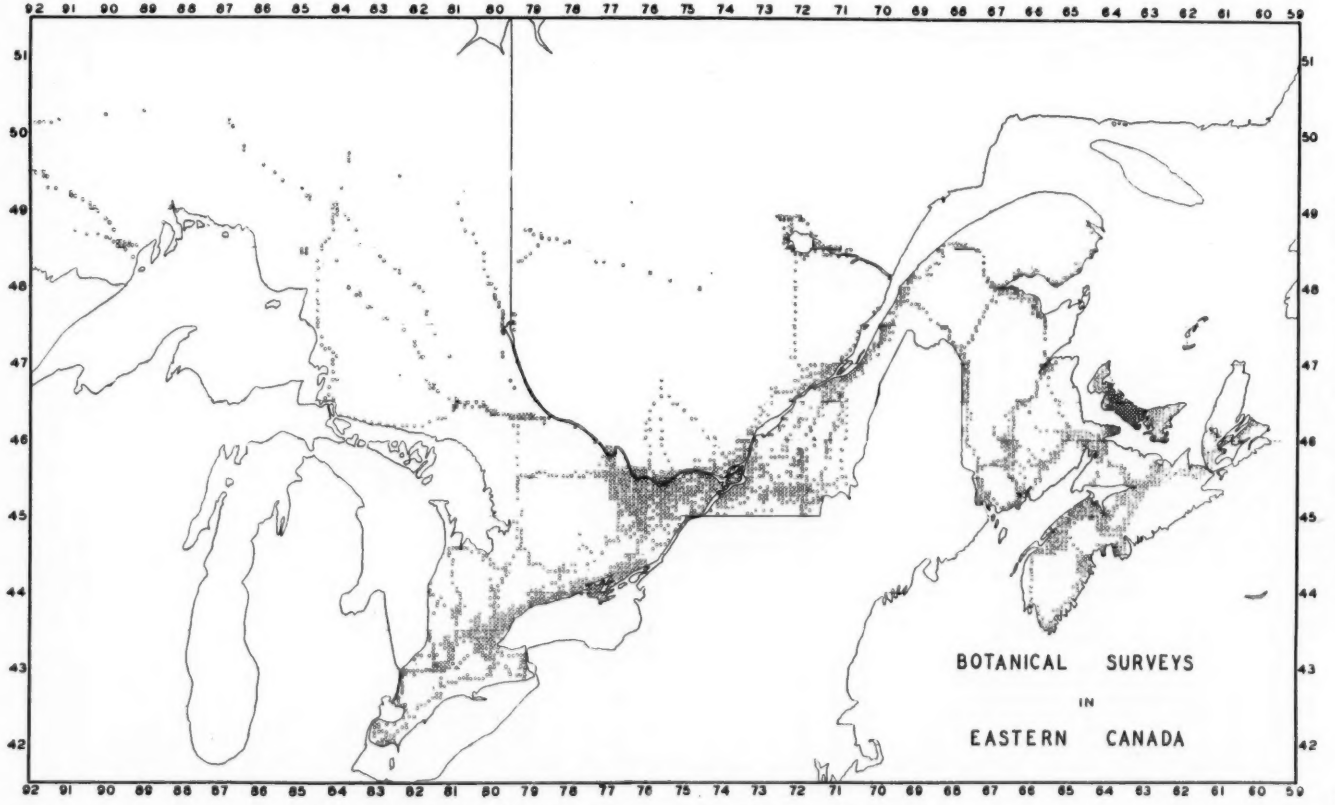


FIG. 6. *Points at which surveys have been made in the Canadian Weed Survey.*

River, *Taylor et al. 2116* (T); Michipicoten Harbour, *Hosie, Harrison & Hughes 170* (T).

A specimen in Nova Scotia Agricultural College Herbarium as follows is on the authority of Prof. A. E. Roland: Sandy river bank, Grand Falls, Victoria Co., N.B.

7b. *Prunus pumila* L. var. *susquehanae* (Willd.) Jaeg.

Ziergehoelze der Garten und Parkanlagen, ed. 1, 400. 1865; Rehder, Man. Cult. Trees and Shrubs, 466. 1927.

Prunus susquehanae Willd. Enum. Pl. Hort. Berol. 519. 1809; Fernald in Rhodora, 25: 73. 1923.

Prunus cuneata Raf. Ann. Nat. 11: 1820; Bailey, Cult. Nat. Plums and Cherries, 63. 1892; Robins. & Fern. in Gray's Man. ed. 7. 499. 1908; Britt. & Brown Illus. Fl. ed. 2, 2: 326. 1913; Wight, Nat. Am. Sp. Prunus, 67. 1915 (U.S. Dept. Agr. Bull. 179); Hedrick, Cherries of N.Y. 35. 1915;

Prunus pumila L. var. *cuneata* (Raf.) Bailey, Cycl. Am. Hort. 1451. 1901.

Specimens examined:

ONTARIO: Northumberland Co., *Macoun*, May 12, 1868 (McG), only foliage, and approaching, if not this variety; St. Williams, *Richardson*, 1919 (AA); Chatham, *McConnell*, June, 1876 (McG); Rondeau Park, *Faull*, June 29, 1905 (T); Sarnia, *Roy. Can. Inst.*,? May, 1925 (T); Bruce Peninsula, Big Andrew Lake, quaking margin, *Krotkov 10638* (G, T); Mud Lake, near Dyer's Bay, *Krotkov 10639* (G, T), Queenleen Lake bog, *Krotkov 10640* (G, T), Mud Lake, north of Emmett Lake, *Krotkov 10641* (T), Little Andrew Lake bog, *Krotkov 10642* (T), shrubby border of Moose Lake, *Krotkov 10643, 10644* (T), Conly Lake, gravelly shore, *Krotkov 10645* (G, T), Lymburner Lake shore, *Krotkov 10646* (T); North Bay, *Marie-Victorin, Rolland-Germain & Meilleur 45342* (M, T); Wanapitae, *Fernald & Pease 3394* (G, T); Espanola, *Fernald & Pease 3395* (G, US, Can, T).

Section 4. *Eucerasus* Koehne

8. *Prunus avium* L.

Fl. suec. ed. 2, 165. 1755; Robins. & Fern. in Gray's Man. ed. 7, 479. 1908; Britt. & Brown, Illus. Fl. ed. 2, 2: 327. 1913; Hedrick, Cherries of N.Y. 28. 1915 (see this for full synonymy); Rehder, Man. Cult. Trees and Shrubs, 471. 1927.

Common name: Mazzard, Sweet cherry.

Distribution: Europe and Western Asia; cultivated and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Barton, *Jack 3181* (AA), naturalized; Wolfville, *Logan*, May 7, 1913 (A).

ONTARIO: Queenston Heights, *Macoun 34,448* (Can, T), *Scott*, May 24, 1895 (T); W. of Niagara-on-the-Lake, *Dearness*, Aug., 1891 (Can).

9. *Prunus Cerasus* L.

Sp. Pl. 474. 1753; Robins. & Fern. in Gray's Man. ed. 7, 499. 1908; Britt. & Brown, Illus. Fl. ed. 2, 2: 327. 1913; Hedrick, Cherries of N.Y. 24. 1915 (see this for full synonymy); Rehder, Man. Cult. Trees and Shrubs, 472. 1927.

Common name: Sour cherry.

Distribution: Western Asia to southeastern Europe; cultivated and escaped in Eastern Canada.

Specimens examined:

NOVA SCOTIA: Barton, *Jack 3182* (AA), naturalized.

PRINCE EDWARD ISLAND: Brackley Point Road, *Fernald, Long, & St. John 7677* (AA, Can, MV).

QUEBEC: Ste. Anne de la Pocatière, *Campagna 8035* (SteA).

ONTARIO: Niagara, *Scott*, May 24, 1897 (Can).

Section 5. *Mahaleb* Focke

10. *Prunus mahaleb* L.

Sp. Pl. 474. 1753; Bailey Cycl. Am. Hort. 1451. 1901; Robins. & Fern. in Gray's Man. ed. 7, 498. 1908; Britt. & Brown. Illus. Fl. ed. 2, 2: 328. 1913; Hedrick, Cherries of N.Y. 31. 1915 (see this for synonymy); Rehder, Man. Cult. Trees and Shrubs 473. 1927.

Common name: Mahaleb cherry.

Distribution: Europe and western Asia; cultivated as a stock for grafting and escaped in Eastern Canada.

Specimens examined:

ONTARIO: Queenston Heights, *Macoun*, May 18, 1901 (Can), *Scott*, May 17 and July 23, 1902 (T); Foster's flats, Niagara, *Macoun*, May 16, 1901 (T); Hamilton, *Dickson*, May 15, 1895, July, 1898 (Can).

11. *Prunus pennsylvanica* L. f.

Syst. ed. 31. Suppl. 252. 1781; Sargent, Silva N. Amer. 4: 35. pl. 156. 1892 (which see for full synonymy); *Robins.* & *Fern.* in Gray's Man. ed. 7, 498. 1908; *Britt.* & *Brown*, Illus. Fl. ed. 2, 2: 328. 1913; Wight, Nat. Am. Sp. *Prunus*, 60. 1915 (U.S. Dept. Agr. Bull. 179); Rehder, Man. Cult. Trees and Shrubs, 473. 1927; *Marie-Victorin*, Fl. Laurent. 370. 1935.

Common name: Bird cherry, Pin cherry, Wild red cherry.

Distribution: Canada and Northern United States. See Fig. 7.

Specimens examined:

NEWFOUNDLAND: St. John's, *Robinson* & *Schrenk*, Aug. 1, 1894 (G); Conception Bay, near Topsail, *Howe* & *Long 1333* (G); Conception Bay, Western Bay, *Torrey 104* (G); Avalon Pen., Whitebourne, Goose Pond, *Fernald, Long* & *Dunbar 26807;* Bay St. George, *Howe* & *Long 1051* (G); banks of Salmonier River, *Robinson* & *Schrenk 74* (AA, US, Can, McG); New Harbour, *Waghorne*, June 16, 1893 (AA, Q); Barred Islands to Fogo Island, *Sornborger*, Aug. 17, 1903 (G); Barachois Beach, *Kennedy 22* (G); Bay of Islands, Tweed Island, *Fernald, Long* & *Fogg 305* (G); Bay of Islands,

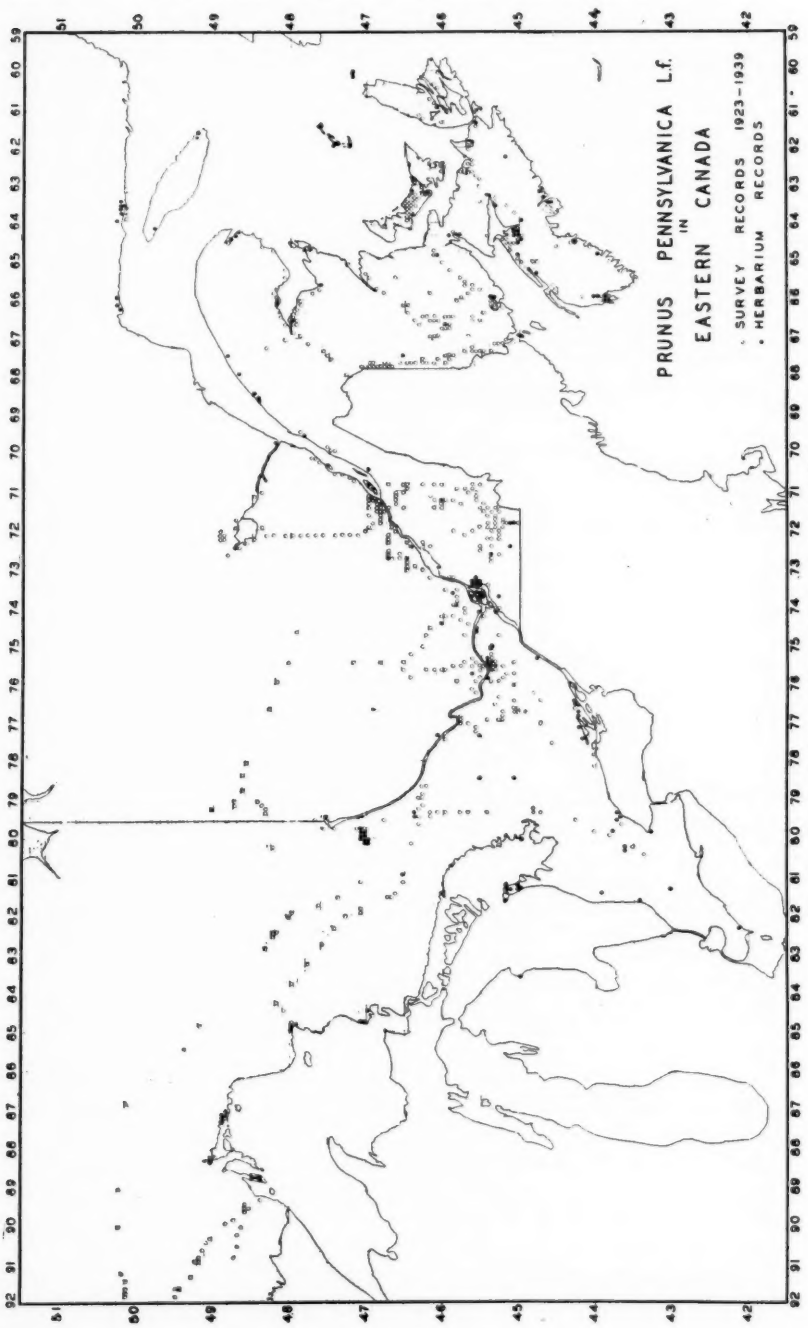


FIG. 7. *Range of Prunus pennsylvanica L.f., Bird cherry.*

Lark Harbour, *Waghorne 40* (G); Teddy Harbour, near Bonne Bay, *Bishop 395* (G); St. Pierre, chemin de l'Anne à Dinent, *Arsène 312* (G); Hamilton River, Labrador, *Low*, July 5, 1894 (Can); Mulligan River, Lake Melville, *Wetmore*, July 27, 1921 (Can).

NOVA SCOTIA: St. Paul Island, between Lena Lake and Petrie's Pond, *Perry & Roscoe 263* (AA, G, Can); St. Paul Island, Top of Coggin Mt., *Perry & Roscoe 262* (G, Can); Ingonish, exposed slopes of Mt. Francy, *Nichols 1700* (G); Baddeck, *Macoun*, July 12, 1898 (Can); Canso, *Fowler*, July 26, 1901 (Q); Caledonia, *Faribault*, June 21, 1885 (M); Fraser's Mills, Antigonish, *Christie*, June 7, 1937 (A); Truro, *Burgess*, June 13, 1883 (T), *Jack 629* (AA); Halifax, *Macoun*, June 10, 1883 (Can), *Burgess*, June 20, 1883 (T); Mumford Road, Halifax, *Bell*, May 27, 1934 (Dal); Halifax Co., *King*, May 25, 1901 (Dal); Armdale, Halifax Co., *Facey*, June 14, 1934 (Dal); Armdale (Dutch Valley), *Fernald, Bartram & Long 24055* (G); Dartmouth, *Fernald, Bartram & Long 24054* (G); Newport, *Dill*, June 2, 1894 (AA); Five Mile River, Hants Co., *Pease & Long 21684* (G); Wolfville, *H. G. Perry*, May 15, 1911, May 21, 1911, May 21, 1912, *Wetmore*, June 9, 1920, *Mason*, May 26, 1919, *Rand*, Oct. 12, 1925 (A); near White Rock, *H. G. Perry*, May 22, 1913 (A); Kentville, *Fyles*, July 29, 1915 (DAO); Waterville, *Chute*, June 15, 1916 (A); Paradise, *Longley*, June 26, 1919 (A); Round Hill, *Jack 3195* (AA); Digby Co., Cedar Lake, *Jack 3765* (AA, G); Central Port Mouton, *Fernald, Bissell, Graves, Long, & Linder 21685* (AA, G); Yarmouth, *Macoun*, May 23, 1910 (Can); *Pease & Long 21683* (G); Brazil Lake, Yarmouth Co., *Bartram & Long 24053* (G); Pleasant Valley, Yarmouth Co., *Jack 3153* (AA).

PRINCE EDWARD ISLAND: Mt. Stewart, *Fernald, Bartram, Long & St. John 7676* (AA, G, US, Can, MV); Brackley Point, *Macoun*, June 22, 1888 (Can).

NEW BRUNSWICK: Sackville, *Goodwin*, June, 1893 (Q); Moncton, *L. M. Perry*, May 28, 1920 (A); Rothesay, *Matthews*, July 6, 1879 (T); Lily Lake (near St. John), *Hay*, June 9, 1876 (NBM); Campbell's Island, *Smith*, July 17, Aug. 20, 1888 (US); Fredericton, *Jack 586* (AA); Fielding, *E. Richardson*, May 25, 1923 (A); Campbellton, *Chalmers*, June 13, 1876 (Can).

QUEBEC: Magdalen Islands, Amherst Island, *St. John 1902* (G); Magdalen Islands, Grindstone Island, *Fernald, Bartram, Long & St. John 7675* (G); Iles de la Madeleine, "La Montagne", Ile du Havre au Ber, *Marie-Victorin & Rolland-Germain 9511* (US, M, MV); Magdalen Islands, Ile Brion, *Marie-Victorin & Rolland-Germain 9510, 9749* (M, MV); Anticosti, Rivière du Renard, *Marie-Victorin & Rolland-Germain 27246* (M), *27384* (AA, Can, M, MV); Anticosti, Potato River, *Adams*, Aug. 3, 1936 (DAO); Sept-Iles, La Grosse-Boule, *Marie-Victorin & Rolland-Germain 18701* (M, MV); Pointe-aux-Esquimaux, *Marie-Victorin & Rolland-Germain 18702* (M, MV, US); Eskimo Point, *Townsend*, June, 1909 (G); Mingan, Seign., *St. John*, Sept. 18, 1895 (Can); Matamek River district, *Bowman 49, 94,*

236 (G); Manowin Island, Seven Islands, Saguenay Co., *Lewis*, Sept. 12, 1928 (Can); Betchewan, Saguenay Co., *Lewis*, Aug. 26, 1928 (Can), *Abbe 1142* (G); Gaspé Bay, *Jack*, Aug. 23, 1927 (AA); Gaspé Basin, *Fowler*, Aug. 16, 1905 (Q); Mont Saint Pierre, Gaspé Co., *Marie-Victorin*, *Rolland-Germain* & *Jacques 33198* (AA, M, MV); Maguasha, *Fowler*, July 5, 1905 (Q); Carleton, *Adams*, Aug. 18, 1935 (DAO); Matane near the Matane River, *Forbes 2022* (G); Little Metis, *Fowler*, Aug. 17, 1906 (G, Q); Rimouski, *Lemay*, 1914 (SteA); Bic, *J. Rousseau 26331, 30006* (M, MV); Ilets Rousseau (Temis.), *Campagna*, *Caron* & *Rousseau*, Aug. 18, 1927 (SteA); Tadoussac, *Kennedy*, Aug., 1892 (G); Ste. Anne de la Pocatière (Kam.), *Campagna*, May, 1931 (SteA); St. Felicien, Shanusham Falls, *Kennedy*, July 18, 1892 (G); Montmagny, *J. Rousseau 20891* (M); St. Famille de l'Ile d'Orleans, *Rousseau 24664, 25247* (M); Montmorency Falls, *Macoun*, July 1, 1905 (Can); Charlesbourg, *Michel*, June 1, 1933 (M); Black Lake, Cariboo Hill, Megantic Co., *Fernald* & *Jackson 12116* (G); Thetford, *Marie-Victorin 11191* (MV, M); vicinity of Trois Rivières, *Stanislas 449* (M); Ste. Maurice River, 10 mi. above Grandes Piles, *Jack*, Sept. 24, 1896 (AA); St. Louis de France, Champlain Co., *Adrien 1634* (AA, M); Lake Knowlton, near Brome, *Knowlton*, May 27, 1923 (G); North Hatley, *Jack*, Sept. 26, 1914 (AA); Farnham, *Jack*, June 2, 1914 (AA); Chateauguay Co., *Jack 3931* (AA); Montreal, Mont Royal, *Marie-Victorin* & *Rolland-Germain 34181* (M, AA, MV), *Majoric 20885* (M), *Mignault*, May 4, 1878 (M); Mont Ste. Anne, environs Montreal, *Marie-Victorin 20890* (M); Longueuil, *Marie-Victorin 4329* (MV, AA, US), *188* (US, MV), *8295* (MV), *11189* (M), *Rolland-Germain 29419* (M, MV); Nichols, Montreal, *Holmes*, May 22, 1821 (McG); La Trappe, *Louis-Marie 24206* (M); L'Epiphanie, *Louis-Marie*, Aug. 5–7, 1930 (Can); St. Jerome, *Marie-Victorin 668* (MV); St. Jovite, Lac Ouimet, *Churchill* (G); Farm Point, *Macoun 87174* (Can); Blueberry Point, Aylmer, *Macoun 59915* (Can); north end of Durant Lake, Atik River, *Wilson*, Sept., 1907 (Can); Eastmain, east coast James Bay, *Potter 488* (G), open woods.

ONTARIO: 5½ mi. southeast of Hawkesbury, near Little Rideau Creek, *Minshall 1500*, fairly common (DAO); 3 mi. east of Hawkesbury, *Minshall 1563* (DAO); Ottawa, *Ami*, May, 1879 (McG), *Fletcher*, May 21, 1879 (McG), Billings Bridge, *Fletcher*, May 15, 1878 (DAO), *Harrington*, May 21, 1906 (Can), Rockcliffe, *Scott*, May 28, 1892 (T), *Macoun*, May 19, 1911 (Can), *E. G. Anderson*, May 19, 1932 (OAC); Prescott, *Billings*, May 20, 1862 (OAC); Jones Falls, *Fowler*, May 20, 1891 (Q); Killaloe, *Milton Jack*, 1903 (AA); Newburgh, *Fowler*, May 14, 1897 (US); Plevna, *Fowler*, Aug. 5, 1902 (G, Q); Hastings Co., *Macoun*, May 12, 1870 (McG); Kingston, *Fowler*, July 23, 1900 (Q), May 23, 1884 (US); Redstone Lane, Haliburton Co., *Krotkov*, Sept. 19, 1929 (T); Humber Plains, *Scott*, May 9, 1896 (Can); Belleville, *Macoun*, May 24, 1871 (Can); Toronto, *Turner*, June 3, 1930 (T); vicinity of Edmonton, *White*, May 17, 1890 (McM); Snelgrove, *White*, May 21, 1898 (Can); Guelph, *Simmons* & *Van Loon*, May 18, 1936 (OAC),

Hart, May 22, 1930 (OAC); Hamilton, *Burgess*, May 21 and Aug. 17, 1889 (McM); Lily Pond Mills, London, *Millman*, May 23, 1884 (T); Aylmer, *R. T. Anderson*, May 23, 1898 (T); Aylmer, *R. T. Anderson*, May 23, 1898 (T); Wingham, *Morton*, June 1890 (Can, OAC); Johnston Harbour, Lake Huron, *Macoun*, Aug. 22, 1901 (Can); Georgian Bay Biological Station, no other data (Can); Port Cockburn, Muskoka, *Burgess*, Aug. 26, 1882 (T, McG); Bruce Peninsula, Stokes Bay, *Krotkov 9146* (US, T, G), *9147* (US, T), Lion's Head, *Watson*, Aug. 7, 1926 (T), Emmett Lake, *Krotkov 10649* (T), Dyer's Bay, *Krotkov 10648* (T), Cabot Head, *Krotkov 9842*, *9843* (T), Tobermory, *Krotkov 7532* (T); Muskoka, *R. W. Smith*, no other data (McM); Chalk River, Ottawa Valley, *Macoun*, May 30, 1884 (Can); Cache Lake, Algonquin Park, *Macoun*, May 30 and July 17, 1900 (Can); North Bay, *Miller*, Aug. 21, 1896 (G), *Ricker*, June 4, 1926 (T); clay belt region, *Tunstell*, 1910 (T); Bear Island, Timagami F. R., *Krotkov 5406*, *5407* (T), *5745* (G, T); Devil's Lake, Timagami F. R., *Krotkov 5408* (T); Timagami Forest Reserve, *Watson*, June 23, 1922 (Can); Timagami Forest Reserve, *Watson 712?*, *5752*, *6841*, *6842* (T); Twin Islands, Timagami region, *E. & D. M. Anderson 26041* (G); Mamainse Mt., Algoma district, *Taylor, Losee & Fitzpatrick 2110* (T); Batchewana Bay, *Taylor, Losee & Fitzpatrick 2111* (T); Mamainse Pt., *Taylor, Losee & Fitzpatrick 2112* (T); Sawyer Bay, *Taylor, Losee & Bannan 1251*, *1252* (T); Shangoina Island, *Taylor, Losee & Bannan 1253*, *1254* (T); Killarney, *Scott*, May, 1895 (T); Nipigon, Lake Superior, *Macoun*, June 23, 1884 (Can); Michipicoten Harbour, *Hosie, Harrison & Hughes 165*, *166*, *167* (T); Schreiber, *Hosie, Losee & Bannan 286*, *288*, *289*, *290* (T).

Specimens in Nova Scotia Agricultural College Herbarium as follows are on the authority of Prof. A. E. Roland: Mixed woods on the ridge above Wolfville; Blomidon; both Kings Co., N.S.

Records from Canadian Weed Survey: These together with the above citations are to be seen in Fig. 7.

SUBGENUS IV. *Padus* Koehne

12. *Prunus serotina* Ehrh.

Beitr. 3: 20. 1788; Sargent, Silva N. Amer. 4: 45. pl. 159. 1892 (which see for full synonymy); Robins. & Fern. in Gray's Man. ed. 7, 497. 1908; Fernald in Rhodora, 18: 140. 1916; Rehder, Man. Cult. Trees and Shrubs, 474. 1927; Marie-Victorin, Fl. Laurent. 321. 1935.

Padus virginiana (L.) Mill. Gard. Dict. ed. 8. No. 3. 1768; Britt. & Brown, Ill. Fl. ed. 2, 2: 329. 1913.

Common name: Black cherry, Rum cherry.

Distribution: Eastern Canada and United States. See Fig. 8.

Specimens examined:

NOVA SCOTIA: St. Croix, *Jack 3324* (AA, G), *3693* (AA); Newport, *Jack 728* (AA); Kentville, Agricultural Experiment Station, *McLellan*, June 20, 1939 (DAO); west of Bridgewater, *Fernald & Long 24051* (G);

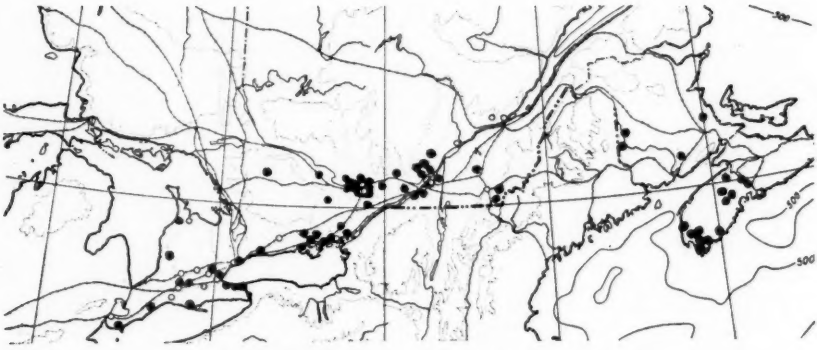


FIG. 8. *Range of Prunus serotina Ehrh., Black cherry.*

New Germany, *Hamilton*, June, 1891 (Can); Bridgewater, *Macoun*, July 15, 1910 (Can); Meteghan, *Fernald & Long 21676* (AA, G); Clyde River, *Jack 3449* (AA, G); Belleville, *Long & Linder 21677* (G); Tusket Falls, Tusket River, *Fernald, Bissell, Graves, Long & Linder 21678* (G, Can); *Argyle, Jack 3428* (AA); Pleasant Valley, *Jack 3135* (AA).

NEW BRUNSWICK: Bass River, *Fowler*, 1874 (Q); Norton, *Hay*, June 11, 1878 (NBM); Florenceville road to Centreville, *H. G. Perry*, June 23–30, 1910 (A); Tracey Mills, Carleton Co., *H. G. Perry*, July 13, 1910, July 21, 1910 (A).

QUEBEC: Danville, *Marie-Victorin, Rolland-Germain & Meilleur 43325* (M), seedlings from border of woods unsupported by typically mature specimens; St. Paul, Arthabaska Co., *Campagna*, July 20, 1922 (SteA), vegetative shoot only; environs Longueuil, *Marie-Victorin 9509* (M), *Marie-Victorin & Rolland-Germain 46755* (T); Ste. Clothilde, *Jack*, May 30, 1904 (AA); Chateauguay, *Marie-Victorin 28325* (M, MV); Montreal, *Lyman*, June, 1879 (McG); Bois des Filion, *Marie-Victorin 28316* (AA, M, MV); St. Janvier, *Jack*, June 2, 1904 (AA); St. Timothée, *Jack*, Sept. 19, 1906 (AA); St. Jerome, *Mignault*, June 1, 1878 (M); vicinity St. Jerome (Laurentides), *Marie-Victorin 3165* (MV); Rawdon, *Marie-Jean-Eudes 702* (M); Hull, *Macoun*, June 1, 1903 (DAO, Can); near Hull cemetery, *Macoun*, Aug. 29, 1874 (Can); Chelsea, *Fletcher*, no date (DAO), *Harrington*, Sept. 11, 1909 (Can); King Mountain (near Hull), *Marie-Victorin 15537* (M, MV); Deschenes, *Rolland 10196* (M, MV); Lake Deschenes on Ottawa River, *Rolland, 15536* (M, MV); Kirk's Ferry, *Harrington*, May 27, 1906 (Can).

ONTARIO: 2 mi. west of Vankleek Hill, *Minshall 1418* (DAO); Ottawa, *Macoun*, Aug. 30, 1911 (Can); *Scott*, June 11, 1931 (Can); May 31, 1892, June 1, 1892 (T), *E. G. Anderson*, June 6, 1933 (DAO); Ottawa West, *Minshall*, May 28, 1935, *Groh*, June 7, 1935 (DAO); Spencerville, *Minshall 873* (DAO); Navan, *Macoun*, Sept. 4, 1911 (Can); Almonte, Forsythe Farm, *Minshall 1747* (DAO); Renfrew Co., Bromley Twp., Con. V, 4 mi. north-

east of Douglas, *Minshall 1750* (DAO); Kingston, *Fowler*, Sept. 8, 1900 (Q); Gananoque, *Fowler*, Aug. 18, 1887 (Q); Collin's Bay, *Fowler*, June 11, 1902 (G, Q); Belleville, *Macoun*, June 10, 1878 (Can); Hastings Co., Sidney Twp., 1½ mi. northwest of Chatterton, *Minshall 602* (DAO); Hastings Co., *Macoun*, June 24, 1871, May 7, 1873 (McG); Bayside, (Hastings Co. ?) *Faull* (T); Battersea, *Fowler*, May 28, 1904 (Q); Hampton, *Allin*, June 18, 1927 (T); Toronto, *Scott*, May 20, 1896 (Can); Guelph, *Dept. of Botany*, June 7, 1929, *Minshall*, June 7, 1932 (OAC); Hamilton, *Senn*, June, 1932 (McM); Lincoln Co., *McCalla*, June 5, Sept. 24, 1897 (Can); 2 mi. west of Niagara, *Dearness*, Aug., 1891 (Can); Foster's Flats, Niagara, *Scott*, May 24, 1898 (Can); St. Williams, *Richardson*, Sept. 5, 1919 (AA); London, *Millman*, May 27, 1881 (T), *Burgess*, May 28, 1878, Aug. 20, 1881 (McG); Aylmer, *R. T. Anderson*, May 24, 1899 (T); Leamington, *Macoun*, May 25, 1901 (Can); Wingham, *Morton*, June 3, 1891 (Can); Red Bay, Bruce Peninsula, *Watson*, Aug. 29, 1926 (T); Cache Lake, Algonquin Park, *Macoun*, June 7, 1900 (Can).

A specimen in Nova Scotia Agricultural College Herbarium as follows is on the authority of Prof. A. E. Roland: Thicket, Clyde River, Shelburne Co., N.S.

13. *Prunus Padus* L.

Sp. Pl. 473. 1753; Rehder, Man. Cult. Trees and Shrubs, 475. 1927 (which see for synonymy).

Common name: European bird cherry.

Distribution: Europe and northern Asia; rarely naturalized in Eastern Canada.

Specimens examined:

ONTARIO: Ottawa, Rideau Canal, *Minshall*, May 15, 1934 (DAO), Dow's Swamp, *Minshall*, May 22, 1935 (DAO); doubtless escaped in both cases from the Dominion Arboretum plantings.

14. *Prunus virginiana* L.

Sp. Pl. 473. 1753; Sargent, Silva N. Amer. 4: 41, pl. 158. 1892 (which see for full synonymy); Robins. & Fern. in Gray's Man. ed. 7,498. 1908; Fernald in Rhodora, 18: 140. 1916; Rehder, Man. Cult. Trees and Shrubs, 476. 1927; Marie-Victorin Fl. Laurent. 322. 1935.

Padus nana (Du Roi) Roemer, Arch. 1 (2): 38. 1797; Britt. & Brown, Illus. Fl. ed. 2, 2: 329. 1913.

Common name: Choke cherry.

Distribution: Eastern and Central Canada and North-Central and Eastern United States. See Fig. 9.

Specimens examined:

NEWFOUNDLAND: Gravelly beach of Rushy Pond, valley of Exploits River, *Fernald & Wiegand* assisted by *H. T. Darlington*, Aug. 28, 1911 (Can); Laurentian area at head of Exploits River system, Little Red Indian Lake, *Fernald & Wiegand 5732* (AA); woods along the Salmonier River, *Robinson &*

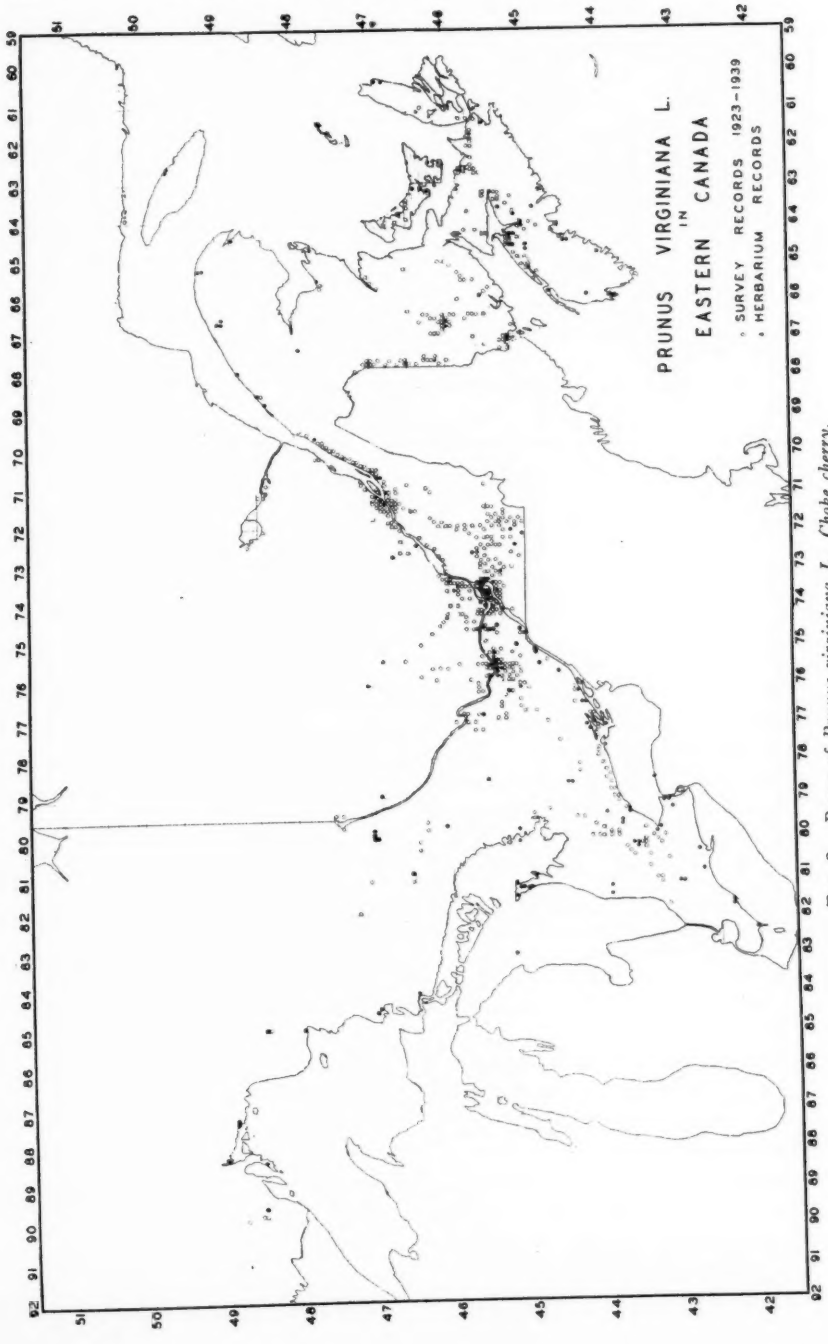


FIG. 9. *Range of Prunus virginiana L., Choke cherry.*

Schrenk 75 (AA, Can, McG); Bay of Islands, Birchy Cove, *Waghorne*, June 20, 1895 (AA, Q); Bay of Islands, Mt. Musgrave and Humber mouth, *Fernald & Wiegand 3626* (G).

NOVA SCOTIA: Valley of Barachois River, *Nichols 1363* (G); Big Intervale, Margaree, *Macoun*, July 18, 1898 (Can); Port Bevis, *Fernald & Long 21682* (G), open woods about base of gypsum cliff; Fraser's Mills, Antigonish, *Christie*, June 16, 1935 (A); Boyleston, *Hamilton*, June 1, 1890 (Can); Pictou, *Jack 783* (AA); Truro, *Jack 633* (AA); Armdale, Halifax Co., *Bell*, June 14, 1934, *Facey*, June 14, 1934 (Dal); Calkin's Farm, Windsor Road, Halifax Co., *Lawson*, June 26, 1891 (AA); Windsor, near Wentworth gypsum quarries, *Fernald, Bartram & Long 24052* (G); Newport, *Dill*, June 2, 1894 (AA); Five-mile River, Hants Co., *Pease & Long 21680* (G); West Gore, Hants Co., *Wallace*, June 15, 1932 (A); Black River, King's Co., *Wetmore*, June 12, 1918 (A); Paradise, *Longley*, June 10, 1919 (A); Black River Falls, *J. H. Perry*, June 3, 1932 (A); Wolfville, *King*, June 8, 1901 (Dal), *H. G. Perry*, June 5, 1912, June 18, 1913, *Mason*, June 10, 1919 (A); Kentville, *McLellan*, June 6, 1939 (DAO); Waterville, *Chute*, June, 1915 (A); Paradise, *Longley*, June 10, 1919 (A); Bridgewater, *Jack 3512* (G, AA), *Macoun*, July 25, 1910 (Can); Port Mouton, *Bissell & Graves 21681* (G, Can); roadside near Digby, *Howe & Long 285* (G); Corberrie, *Jack 3123* (AA); Tusket, *Long & Linder 21679* (AA, G).

PRINCE EDWARD ISLAND: Charlottetown, *Fernald, Long & St. John 7674* (G); Brackley Point, *Macoun*, Aug. 31, 1888 (Can); Malpeque, *Fowler*, July 14, 1904 (Q).

NEW BRUNSWICK: St. Andrews, *Fowler*, June 18, July 10, 1900 (Q); Fredericton, *Fowler*, Aug. 4, 1880 (Q), *Sargent*, Aug. 2, 1912 (AA); Woodstock, *Fernald & Long 13940* (G); Bathurst, *Meehan*, May, 1880 (McG); Restigouche River, *Chalmers*, June 17, 1876 (Can); Tracey Mills, *H. G. Perry*, July 13, 1910 (A); Hampton, *Hay*, June 10, 1876 (NBM); Emerson, *Fowler*, July 14, 1887 (Q).

QUEBEC: Grosse Island, Magdalen Ids., *Fernald, Long & St. John 7673* (G); Brion Island, Magdalen Ids., *St. John 1906* (G); Rivière Vaureal, Anticosti, *Marie-Victorin, Rolland-Germain & Louis-Marie 20889* (M, MV), *Marie-Victorin & Rolland-Germain, 27370* (Can, M, MV); Douglastown, *Collins, Fernald & Pease 6407* (G); between Lac Mont Louis and north fork of Madeleine River, Gaspé Co., *Fernald, Dodge & Smith 25869* (G, Can) Nettle Gully, alt. 400 m., northern base of Mt. Collins, *Fernald, Griscom, Pease & Smith 25867* (G, Can, MV); northern base of Mt. Mattaouisse, *Fernald, MacKenzie & Smith 25868* (G, Can, MV); woods at about 500 m. alt. in Fernald Basin between Mts. Mattaouisse and Fortin, *Fernald & Smith 25870* (G, Can, MV); Metis Park? *Baylis?*, May 30, 1885 (T), specimen with bifurcated inflorescence; Bic, *Forbes*, June 25, 1905 (Can); Cap Enrage (Rimouski Co.), *J. Rousseau 26488* (MV); Ste. Anne (Kam.), *Campagna*, June 1931 (SteA); Chicoutimi, *Marie-Anselme*, June 15, 1933 (M); Grand'Mere, *Adrien 1665* (MV); Sainte-Marie, Champlain Co., *Stanislas*

447 (M); Montmorency Falls, *Macoun*, July 3, 1905 (Can); Berthier en Bas, *J. Rousseau 26236* (Can, M, MV)), *Marc Rousseau 30066* (M, MV); Wolfe's Cove below Quebec, *Sargent*, Aug. 4, 1912 (AA); Plains of Abraham (Quebec), *Romeo 1766* (M); Levis, *Jack*, June 1, 1903 (AA); Phillipsburg, *Knowlton*, May 24, 1925 (G); Massawippi, *Jack*, Sept. 26, 1914 (AA); Stanstead, *Jack*, June 2, 1914 (AA); St. Dominique, *Marie-Victorin* & *Rolland-Germain 34041* (M), *34042* (M, MV); Longueuil, *Marie-Victorin*, *9475*, *11190* (M), *Rolland-Germain 29433* (M), *Marie-Victorin 277*, *278*, *667* (MV); Montreal, *Lyman*, May, 1879 (McG), *Mignault*, May 10, 1878 (M); Nichol's (Montreal), *Holmes*, May 29, 1821 (McG); St. Laurent, *Adrien 999* (M); Mont Royal, *Marie-Victorin 21735* (M, MV); Ile Bizard, *Adrien 1700* (M, MV) *1701* (MV); Ste. Anne, Montreal Isd., *Jack*, May 29, 1913 (AA); Highlands, *Jack*, May 21, 1900 (AA); Verdun, *Jack*, May 30, 1902 (AA); La Trappe, *Louis-Marie 2482* (M); Grenville, *Mignault*, May 1, 1878 (M); L'Epiphanie, *Louis-Marie*, *Dudemaine* & *Laporte 1548* (Can); Ste. Agathe des Monts, *Jack*, May 29, 1903 (AA); Rivière Ouareau, Rawdon, Montcalm Co., *Marie-Jean-Eudes 556* (M); Hull, *Harrington*, May 26, 1906 (Can); Aylmer, *Harrington*, June 3, 1905 (Can); environs of Ottawa, *Marie-Victorin 9129* (M); Fort Temiscamingue, *Marie-Victorin*, *Rolland-Germain*, & *Meilleur 43948* (M).

ONTARIO: 5½ mi. southeast of Hawkesbury, near Little Rideau Creek, *Minshall 1501* (DAO); 1 mi. west of Vankleek Hill, *Minshall 1383*, roadside fencerow common (DAO); Kirkhill, *Minshall 1295* (DAO); Lochiel, *Minshall 1340*, common (DAO); 6 mi. northeast of Cornwall, Leitch farm, *Minshall 1241* (DAO); Cornwall, Flanagan's Point, *Jack*, May 29, 1914 (AA); 2½ mi. southeast of Finch, Eligh farm, *Minshall 1176* (DAO); 2 mi. east of Iroquois, *Minshall 1213*, *1214* (DAO); Navan, *Minshall 1613* (DAO); Spencerville, *Minshall 867* (DAO); Ottawa, Rockcliffe Park, *Macoun*, May 21, 1903 (Can, DAO), May 19, 1911 (Can), Billings Bridge, *Fletcher* (DAO), Canal Road, *Minshall*, May 22, 1937, May 28, 1935, May 27, 1937 (DAO), Dow's Swamp, *Harrington*, May 30, 1906 (Can); Ottawa West, *Minshall*, May 27, 1937 (DAO); west of Ottawa, *Macoun 87176* (Can); Lyn, *Minshall 839* (DAO); Almonte, Forsythe farm, *Minshall 1642*, common along fencerows (DAO); Carleton Place, *Macoun*, May 31, 1884 (Can); Renfrew Co., Bromley Twp., Con. V, 4 mi. northeast of Douglas, *Minshall 1751*, roadside fencerows, common (DAO); Gananoque, *Fowler*, Aug. 24, 1884 (Q); Kingston, *Fowler*, June 2, 1897 (Q); Kingston Mills, *Ford*, May 23, 1894 (Q); Plevna, *Fowler*, Aug. 18, 1902 (G); Picton, *Fowler*, June 3, 1902 (Q); Belleville, *Macoun*, June 15, 1870 (Can), June 1878 (OAC); Lindsay, *Scott*, Aug. 16, 1903 (T); Cameron Lake, Victoria Co., *Krotkov*, Sept. 10, 1929 (T); Toronto, Bathurst St., near Eglinton, *R. W. Smith*, June 2,? (McM); North Toronto, collector not stated, May, 1894 (McM); Edmonton, *White*, May 17, 1890 (McM), May 25, 1891, July 31, 1893 (Can); Aurora, collector not stated, nor date (McM); Cressy, *Oughton*, June 10, 1928 (T); Cartwright, *Scott*, Aug. 11, 1898 (T); Guelph,

Dewar, (OAC), *Hamilton* (T), both without dates, *Doherty*, July 10, 1896 (OAC); Hamilton, *Clappison*, June 21, 1898 (T), May 25, 1900 (T); De Cew Falls, Lincoln Co., *McCalla*, May 22, 1897, July 26, 1897 (Can); Niagara, *Potter*, 1908 (T); Foster's Flats, Niagara River, *Scott*, May 24, 1898 (T); St. Williams, *Richardson*, 1919 (AA); London, *Millman*, May 23, 1881, Aug. 10, 1881 (T), *Burgess*, Aug. 10, 1881 (McG); Aylmer, collector not stated, May 27, 1898 (T); Rondeau Park, *Faull*, May 21, 1915, June 5, 1915 (T); Pt. Pelee, *Macoun*, Aug. 6, 1901 (Can); Wingham, *Morton*, May 28, 1891 (Can); Huron Co., *Fraser*, June 7, 1928 (T); Cape Croker, *Watson 3408* (T); Bruce Peninsula, Umbrella Lake, *Krotkov 9150* (T), Stokes Bay, *Krotkov 9149* (T), Dyer's Bay, *Krotkov 10647* (T); Red Bay, *Watson 3602* (T); Cabot Head, *Krotkov 9841* (T); Devil's Island, Tobermory, *Krotkov 7535* (G, T); Georgian Bay, *Huntsman?*, July 18, 1905 (Can); Cache Lake, Algonquin Park, *Macoun*, June 7, Aug. 11, 1900 (Can); Frank's Bay, Lake Nipissing, *Fallis*, June 11, 1929 (T); Timagami Forest Reserve, *Watson 6843* (G, T) *6844* (T), *Krotkov 5409* (T); Bear Island, Timagami Forest Reserve, *Krotkov 5410* (G, T); Garden River, Algoma Co., *Fassett 14736* (G); Batchewana Village, *Taylor, Hosie, Fitzpatrick, Losee & Leslie 2114* (T); Mamainse Mtn., *Taylor, Hosie, Fitzpatrick, Losee & Leslie 2113* (T); Sawbill Lake, *Taylor, Losee & Bannan 1255* (T); Nipigon, Lake Superior, *Macoun*, June 26, 1884 (Can); Michipicoten Harbour, *Hosie, Harrison & Hughes 168, 169* (T); Schreiber, *Hosie, Losee & Bannan 292, 293* (T).

Specimens in Nova Scotia Agricultural College Herbarium as follows are on the authority of Prof. A. E. Roland: Sandy hillsides, common, south branch of the North R., Colchester Co.; Blomidon, roadside, Kings Co.; roadside, Antigonish; Lornedale near Truro, Bible Hill, Truro, and swamp, Truro, all N.S.

Records from Canadian Weed Survey: together with the above citations are to be seen on Fig. 9.

Analysis of Choke Cherry (*P. virginiana*) and Bird Cherry (*P. pennsylvanica*) Records

Incidence of both choke cherry and bird cherry has been further derived by charting the records taken from the Canadian Weed Survey, the percentage incidence* being charted for each meridian. The 2805 surveys in Eastern Canada are distributed somewhat in keeping with uneven settlement, but their unevenness apparently does not vitiate the results.

From a study of the chart (Fig. 10) in conjunction with the maps the following seem significant deductions:

North and west of Georgian Bay, from rather sparse surveys, *P. virginiana* is almost absent, while *P. pennsylvanica* becomes increasingly and extra-

** The term percentage incidence is used for the numerical value obtained by rendering the number of records for a given species within one meridian into a percentage of the surveys within that meridian.*

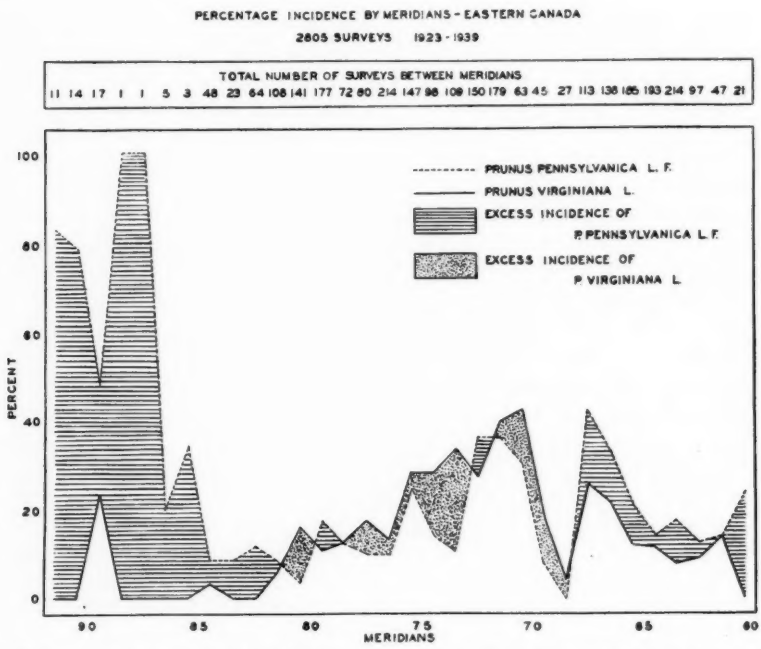


FIG. 10. *Percentage incidence by meridians of Prunus pennsylvanica L.f. and P. virginiana L., in Eastern Canada; 2805 surveys, 1923–1939.*

ordinarily abundant. This region is entirely within the Canadian life zone.

In the Carolinian life zone of southern Ontario both species are comparatively scarce.

From south of Georgian Bay eastward through Ontario and the part of Quebec south of the Canadian life zone *P. virginiana* is dominant. Any high incidence of *P. pennsylvanica* within these meridians is mostly from surveys in the Laurentians to the north, as for instance along the lines from Quebec city to Lake St. John district, and from North Bay to the Abitibi district. The same influence is evident in a small way at the point where the northern belt reaches through to the St. Lawrence at Kingston.

From Meridian 68° to 69° surveys are few, and records of both species are few in number and in percentage incidence. The same situation prevails at a point north of Lake Superior and an increased number of surveys in both cases would be desirable.

From the western boundary of New Brunswick to the Atlantic *P. pennsylvanica* is almost entirely in the ascendency, particularly so where the broken land of eastern Cape Breton, and the prevailingly light soils of Prince Edward Island and of Victoria and Carleton counties in New Brunswick exert their influence. These points are likewise in the Canadian life zone.

Discussion and Conclusions

Prunus species and varieties to the number of sixteen are recognized from within the eastern half of Canada. These include five plums and the peach, three sand cherries, regarded as varieties of one species, and six cherries. Two plums, the sand cherries, and three other cherries are native, the others all escapes from cultivation.

The relative abundance in the wild state of the various species and varieties is fairly well reflected in the volume of citations brought together. Distribution not being the same for each, abundance locally for some may be greater, however, than the total of citations would suggest. Introduced species, likewise, are too sparingly collected to indicate adequately their complete distribution. It is a safe assumption that nowhere do they constitute a large part of the wild *Prunus* population. Planted plums and cherries, subject to the usual orchard protection practices, are not likely either to be major sources of trouble through harbouring insect vectors of disease.

Of the two native plums, *P. americana* is virtually confined to the southwestern peninsula of Ontario, where it is evidently sufficiently abundant in the peach-growing district to be of importance in relation to disease transmittal. *P. nigra* occurs in the Maritimes, Quebec, and Ontario, but in southern Ontario it appears to be near its southern limits, overlapping the northern limits of *P. americana*, and it is doubtful whether it shares much responsibility with the other for any trouble caused the fruit grower. To potato growers in parts of Eastern Canada it may be found to be of importance.

The sand cherries *(Prunus pumila)*, taken together, are quite widely distributed in the area, but of all the *Prunus* are least in proximity to agricultural settlements. Two are largely confined to Ontario shores and wild land; the other, variety *depressa*, occurs from Ontario to the Atlantic. The more upland habitat ascribed by some writers to the variety *susquehanae* as contrasted with the shore-loving *pumila*, may have some confirmation from these Canadian sources, but overlapping along the Great Lakes system is considerable.

Of the three remaining wild cherries, the black cherry has a range well within the northern limits of bird and choke cherry. It occurs only in the more southerly half of Nova Scotia, sparingly in New Brunswick (where some treatments show it absent), and in southern Quebec and Ontario below a line following the St. Lawrence and Ottawa valleys and across to the north of Lake Huron. We have seen no specimens to substantiate reported occurrences west of Lake Superior. Within its range black cherry is to be found in deep soils in woodlands and along fence rows.

Choke cherry and bird cherry have a wide distribution throughout our area and farther west. They are often found growing together, but in a general way it may be said that choke cherry frequents the fencerows and environs of farm land, while bird cherry occupies sandier tracts and broken land and all the wilderness to the north. This difference is clearly revealed in the mapping of the two species at such points as the Laurentian country

between Quebec city and Lake St. John, and the sandy tracts beyond the lake, and along the St. Lawrence river below Quebec, where bird cherry predominates on the rocky north shore and choke cherry is more plentiful in settlements along the opposite side. The rather sparse surveys right across from Lake St. John to Lake Superior are nevertheless numerous enough to show dissimilar incidence of the two species.

A study of the maps shows a few areas where neither species is prominent. At least some of these are similar in their lowland plains aspect, i.e., the Valleyfield-Huntingdon area south of Montreal and the southern tip of Ontario.

These and similar but less obvious places would seem to be the ones to be further surveyed and studied in the search for a region for the production of disease-free foundation stock. The Bay of Chaleur potato-growing district is obviously better situated than the Victoria-Carleton district of the same province. The lower St. Lawrence and the Shipshaw area near Chicoutimi, both of which are being considered as to suitability, appear only moderately safe, but surveys are scarcely sufficient as yet for a pronouncement.

Taking all things into account, choke cherry would appear to be the species most usually present where potato growing would be affected. In some of the lighter soil regions bird cherry would partly take its place. *Prunus nigra* might often be an additional factor.

From the standpoint of geographical distribution and frequency these are the three most abundant species in potato-growing regions. It would now be most interesting to learn whether these species or perhaps the less common species are of most importance as overwintering hosts of the vectors.

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CANADIAN WILTSHIRE BACON

X. DISTRIBUTION OF CHLORIDE[1]

BY W. H. WHITE[2] AND W. H. COOK[2]

Abstract

Highly significant differences in chloride content were observed at different positions across the ham of Wiltshire sides, the portion next to the tank pickle during cure usually containing the most chloride. Of the various factors studied, the number of injections used for pumping a side, and the age from cure, were the only ones found to affect the chloride distribution. Greater uniformity was obtained by increasing the number of injections, and with increasing age from cure. Within the range used in practice for curing Wiltshire sides, the chloride content of the pickles or the number of days in cure was not related to the chloride distribution. An equation is given relating the uniformity of chloride distribution to the number of injections used per side in pumping, and the number of days from the end of cure. This equation indicates that, on the average, the variations in the chloride content within the ham of a Wiltshire side will be reduced to the same magnitude as the variations between sides cured in the same plant, after holding at 1.1° C. for periods of about 12, 26, and 30 days, for sides receiving 27, 8, and 0 injections, respectively.

Introduction

Curing of bacon involves not only the addition of a sufficient quantity of curing salts, but also their distribution throughout the meat. The period of maturation, following removal of the bacon from cure, is believed to improve the quality of the product, particularly with respect to colour uniformity and stability, and the development of the typical bacon flavour. Because of the relatively short curing periods employed in the preparation of Wiltshire bacon, it seems probable that the distribution of salts takes place mainly after removal from cure. Presumably such distribution must reach a certain level of uniformity before the desirable changes involved in maturation can occur. The apparent importance of a uniform salt distribution, both from its direct effect on palatability and its possible indirect effect on maturation, led to a study of this subject on factory-cured Wiltshire bacon.

The content of curing salts and properties of Wiltshire bacon obtained from different Canadian packing plants, and the effect of storage and smoking on these quantities, have been reported in earlier papers of this series (5, 6). The present study was confined to the distribution of chloride throughout the ham

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at the different samplings, with a few additional determinations on the content and distribution of chloride, nitrate, and nitrite, in pumped and unpumped sides.

Since the sides obtained from different plants varied in age from two to 11 days from the end of cure when the first analyses were made, it is obvious that the results do not give a measure of the rate of salt penetration during cure. In commercial practice, moreover, pickle is injected into Wiltshire sides at a number of points before they are placed in the curing tank. This practice, termed pumping, doubtless tends to increase the total salt content and to improve the uniformity of salt distribution, but the variations between the methods employed in different plants could easily mask the effect of differential rates of salt penetration in different carcasses. Factors involved in pumping include the amount of pickle injected, and the position and number of stitches used. Of these quantities, only the effect of the number of stitches could be determined, since the position of the stitches cannot be described in quantitative terms, while the amount of pump pickle added to the sides in question was unknown. It appears, however, that the quantity of pickle retained by the sides is a function of the number of stitches employed (4).

Material, Sampling, and Methods

A description of the sides used in this study, the method of transfer from the plants to the laboratory, and the conditions of storage between samplings, has been reported in the first paper of this series (7). The samples were obtained in the conventional manner (1) from a central strip, about $1\frac{1}{2}$ in. wide, taken from the slice of ham removed for analysis at each sampling (7). These strips included the cross section of the femur, since certain practical operators attach considerable importance to the salt concentration of the meat in the vicinity of the bone. There is also some evidence (3) that the adductor and vastus intermedius muscles do not absorb salt readily. Each strip was divided into three, instead of the five or more sections usually employed in studies of this sort. This practice was followed since the hams on Wiltshire sides are rather small and, being compressible, tend to vary somewhat in thickness, depending on the conditions and method of baling and transport. The position from which each of the samples was taken is shown in Fig. 1. The material from Position 1, on the inside of the ham, was adjacent to the tank pickle during cure; that from Position 2 represented the middle of the ham, surrounding the bone; while Position 3 represented the meat adjacent to the layer of fat and skin, through which little salt is likely to penetrate during cure.

The lean meat was separated from the bone, fat, and connective tissue, and each portion thoroughly mixed and ground by passing it through a food chopper. The samples were then frozen and stored at $-29°$ C., until required for analysis. The chloride content was determined according to the official method of the A.O.A.C. (9, p. 354), in which the sample is ashed, leached with hot water, and the chloride content of this extract found by the Volhard

FIG. 1. *Portions of Wiltshire hams used for determining distribution of chloride.*

procedure. Statistical methods (10) were employed for interpreting the results.

The entire group of 44 sides were analysed at the first and second samplings; i.e., when received at the laboratory, and again after 10 days' storage at 1.1° C. Only 16 of these sides were analysed for chloride distribution at the third sampling, i.e., after smoking. While in subsequent tables the results for the entire group are considered together for the first two samplings, those for the 16 sides analysed three times are also considered separately, in order to provide comparable results over all three samplings.

Mean Chloride Content by Positions and Samplings

The mean age and sodium chloride content by positions and samplings over all sides are given in Table I. The chloride content of the slice of ham from which the chloride distribution samples were taken has already been reported (6). The table is divided into two main sections, the first giving the results for the 44 sides analysed twice and the second the results of the 16 sides analysed three times.

Although the mean age of the entire group of sides from cure was about seven days at the time of the first sampling, it is evident that the sodium chloride content at Position 1, next to the tank pickle, was much higher than at Positions 2 and 3. This gradient still existed, but was much smaller, at the second sampling when the sides were about 18 days old. The group of 16 sides analysed three times indicated essentially the same behaviour at the first two samplings, and also showed that the chloride is quite uniformly distributed after smoking, when the mean age for the group was about 24 days.

The results reported in the last column represent the best estimate of the true chloride content of the hams, since these figures (6) were obtained by grinding and sampling a piece of each ham several times as large as that of the entire strip used in the salt distribution studies. Other investigators (8) have observed that this central-strip sampling procedure does not represent

TABLE I

MEAN SODIUM CHLORIDE CONTENT OF SIDES BY POSITION AND SAMPLING

Sampling	Mean age of sides from cure, days	Position	Mean sodium chloride content		
			By position, %	By sampling, %	From previous analyses (6), %
For all 44 sides—sampled twice					
First	7.4	1	6.42	4.30	3.43
		2	3.45		
		3	3.03		
Second	17.8	1	4.49	3.91	4.42
		2	3.78		
		3	3.46		
For 16 sides—sampled three times					
First	9.9	1	6.87	4.34	3.19
		2	3.47		
		3	2.69		
Second	21.4	1	4.46	3.84	4.19
		2	3.73		
		3	3.33		
Third	24.4	1	4.06	3.99	—
		2	3.90		
		3	4.01		

the true salt content of the ham. The present results indicate that the mean chloride content over all three positions in this central strip apparently overestimates the true chloride content at the first sampling and underestimates it at the second. In more specific terms the chloride content at Position 1 was much above the average at the first sampling, while that at Positions 2 and 3 was still below the average at the second sampling. From this it appears that the positions chosen represent not only those likely to have the highest chloride content initially, but also those that are the last to be affected by the movement of chloride throughout the ham.

The significance of the observed differences in the chloride content between positions at the different samplings was determined by an analysis of the variance for each sampling, into portions attributable to: between positions; between sides from different plants; between sides from the same plant; the interaction positions × plants; and sampling and analytical error. The results, which appear in Table II, show that the difference between sides from different plants is usually significantly greater than the differences between sides from the same plant, and that this in turn usually exceeds the sampling and analytical errors. This merely confirms the findings reported

TABLE II

ANALYSIS OF VARIANCE OF SODIUM CHLORIDE CONTENT AT DIFFERENT SAMPLINGS

Variance attributable to	Sampling					
	First		Second		Third	
	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
For all 44 sides—sampled twice						
Between positions	2	149.926**	2	12.206**	—	—
Between plants	21	4.148	21	3.320**	—	—
Between sides	22	2.202**	22	0.797**	—	—
Differential effect, position × plants	42	1.977**	42	0.433**	—	—
Sampling and analytical error	44	0.674	44	0.177	—	—
For 16 sides sampled for salt distribution—three times						
Between positions	2	79.186**	2	5.262**	2	0.102
Between plants	7	8.129**	7	5.227**	7	7.329**
Between sides	8	0.798**	8	0.583	8	0.844
Differential effect, position × plants	14	1.232**	14	0.343	14	0.250
Sampling and analytical error	16	0.175	16	0.287	16	0.434

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

in an earlier paper (6). The fact that these differences were sometimes not significant at certain samplings in the present study does not indicate disagreement with the earlier results, since they dealt with the average effect over all sides and samplings.

The present study is concerned primarily with the variance between positions, since this quantity reflects the variations in chloride distribution. It is evident that the observed differences in the chloride content at different positions, as indicated by the results in Table I, are statistically significant at the first two samplings. Since the mean square between positions decreases between successive samplings, it is evident that the chloride becomes more uniformly distributed during the maturation period. In the group of sides sampled after smoking, the difference between positions is not significant, indicating the absence of a systematic gradient in chloride concentration.

The interaction position × plants was also significant at both samplings for the entire lot of 44 sides. This indicates that there was not a uniform gradient in chloride concentration from one position to another in the product of all plants. It is possible that this could be attributed to the different pumping practices, etc., followed in different plants. Since the sides from the different plants varied in age from two to 11 days from cure at the time

of the first sampling, it may be that the different gradients merely reflect the effect of ageing.

Chloride Distribution in Relation to Curing Practice

Before attempting to establish a quantitative relation between the chloride distribution and the age of the sides, some consideration had to be given to the effect of variations in the curing practices, in order that these factors could be taken into account. The standard deviation between the positions for both sides received from each plant was used as an estimate of chloride distribution. Although the standard deviations between all three positions were used initially, it was found, as the computations proceeded, that the difference between Positions 1 and 3 was an equally sensitive measure of the variations in chloride content. The standard deviation or difference between the positions observed for all sides at the first sampling was correlated with the known curing factors, as outlined in the first part of Table III. The results of the second sampling were not included, since the influence of age tends to minimize any difference attributable to the various curing practices.

Examination of these correlation coefficients shows that only the coefficient relating the standard deviation between positions and the number of stitches used per side attains the 5% level of significance. In fact, the other coefficients relating quantities involving the number of stitches per side, although not significant, were much larger than the correlations between the standard deviation and other quantities, and probably would have been significant had the sides been analysed immediately after cure. It is therefore concluded that chloride distribution is influenced more by the number of stitches used in pumping than by the observed variations in the chloride content of either of the pickles or by the number of days in cure.

In an earlier paper (6), it was shown that the number of stitches injected per side was the only known curing practice that was correlated with the over-all chloride content of bacon. Since the chloride content at Position 1 should be affected primarily by the tank curing practice, and that at Positions 2 and 3 by the pumping practice, correlation coefficients were computed between the chloride contents at these positions, for the first sampling, and the known properties of the pumping and tank curing practices. The results obtained are shown in the second part of Table III. Although none of the correlation coefficients are significant, their magnitude suggests that the number of stitches per side is the most influential factor of those studied in determining the chloride content at Position 2. It appears, therefore, that within the limits used in practice, similar amounts of chloride are taken up from the tank pickle regardless of its concentration or period in cure. In these circumstances, pumping the sides would be expected not only to reduce the variation in chloride content between the outside and the centre positions, but also to increase the total amount in the bacon. Nevertheless, correlation coefficients failed to demonstrate a definite relation between chloride content and chloride distribution.

TABLE III

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE DISTRIBUTION AND CERTAIN CURING PRACTICES

Quantities correlated	D.F.	r
Sodium chloride distribution in relation to curing practice		
Standard deviation of sodium chloride content at all 3 positions with:		
No. of stitches per side	20	−0.42*
No. of stitches per side × sodium chloride in pump pickle	20	−0.38
Difference in sodium chloride content between Positions 1 and 3 with:		
No. of stitches per side	20	−0.42*
Days in cure	20	−0.14
Sodium chloride in tank pickle	20	+0.01
No. of stitches per side × sodium chloride in pump pickle	20	−0.38
No. of stitches per side, independent of days in cure	19	−0.41
No. of days in cure, independent of number of stitches per side	19	−0.08
Sodium chloride content in relation to curing practice		
Sodium chloride content of meat at Position No. 1 with:		
Days in cure	19	+0.14
Sodium chloride in tank pickle	19	+0.01
Sodium chloride in tank pickle × days in cure	19	+0.14
Sodium chloride content of meat at Position No. 2 with:		
No. of stitches per side	20	+0.37
No. of stitches per side × sodium chloride in pump pickle	19	+0.34
Sodium chloride content of meat at Position No. 3 with:		
No. of stitches per side × sodium chloride in pump pickle	19	+0.07
Relation between distribution and content of sodium chloride		
Standard deviation in sodium chloride content at all 3 positions with mean chloride content of all 3 positions for:		
First sampling by plants	20	−0.18
First sampling by sides	42	−0.11
Second sampling by plants	20	−0.36
Second sampling by sides	42	−0.29

* *Indicates 5% level of significance.*

Comparison of Pumped and Unpumped Sides

The results presented in this and earlier papers (5, 6) have indicated the importance of the method of pumping, in determining the concentration of curing salts in bacon. To confirm this a few preliminary experiments were made on the composition of pumped and unpumped sides cured simultaneously in the same tank in a packing plant. Four pumped and two unpumped sides were sampled and analysed, by the methods previously described (11), immediately on removal from cure and again 17 days later. The mean com-

position of each group of sides, with respect to sodium chloride, nitrate, and nitrite, appears in the first part of Table IV, and the chloride distribution, as shown by the sodium chloride content at the different positions, in the second part. Statistical methods were not applied to these few results, but since the different sides in each group varied considerably, only relatively large differences can be considered significant.

TABLE IV

COMPOSITION OF, AND CHLORIDE DISTRIBUTION IN, PUMPED AND UNPUMPED SIDES

Constituent	Pumped		Unpumped	
	1st sampling	2nd sampling	1st sampling	2nd sampling
Constituent—				
Sodium chloride, %	2.61	3.68	0.56	1.93
Sodium nitrate, %	0.16	0.16	0.008	0.028
Sodium nitrite, p.p.m.	17	34	0.8	27
Sodium chloride distribution				
Position—				
1	4.92	2.96	3.15	2.28
2	3.08	2.91	0.82	1.26
3	3.41	3.48	0.46	1.05

It is evident that the slice of ham analysed at the first sampling contained less of all three salts than at the second sampling. In general, this apparent increase in the content of the curing salts must be attributed to differences between positions rather than a change with time (6). Nevertheless, the proportionately greater increase in the nitrite content of the unpumped sides between samplings suggested a real change during storage. This confirms an earlier finding (6) that nitrite is produced in sides having a low nitrite content initially.

Comparison of the values obtained at the second sampling, when a reasonably uniform distribution of the salts has occurred, indicates that pumping contributes about half the chloride and 80% of the nitrate found in the bacon. The chloride content at the three positions for the second samplings suggests a relatively uniform distribution throughout the hams of the pumped sides, while the unpumped sides still had about twice as much chloride at the position adjacent to the tank pickle, as at the central positions. The importance of pumping in determining both the concentration of the salts and their distribution in the sides is apparent.

Effect of Pumping and Ageing

It was concluded from the results already presented that the number of stitches used for pumping and the age from cure were the principal factors affecting the distribution of salts in hams of Wiltshire sides. In order to obtain

a quantitative estimate of the effect of these two factors, a partial regression equation was computed for the standard deviation between positions on number of stitches used in pumping and the number of days from cure over all samplings. This equation was found to be:

$$Y = 4.64 - 0.074\,S - 0.126\,N$$

where

Y = standard deviation between salt content at different positions,

S = number of stitches used per side

and

N = number of days from cure.

Although this equation shows the extent to which the variations in chloride content between different positions decrease with increase in number of stitches per side and days from cure, it is also important to determine the amount of the total variance accounted for in this way. The mean square for the observed variations in Y (standard deviation between positions) was found to be 1.42, and after subtracting the variance accounted for by the above equation, the residual mean square was 0.70. This shows that variations in pumping practice and age of the sides accounted for about half of the observed variance.

From a practical standpoint, there is no apparent advantage in reducing the variations in salt content within an individual side to less than those between different sides produced under the same conditions in the same plant. Reference to Table II shows that the mean square between sides over all samples at the second sampling was 0.80.

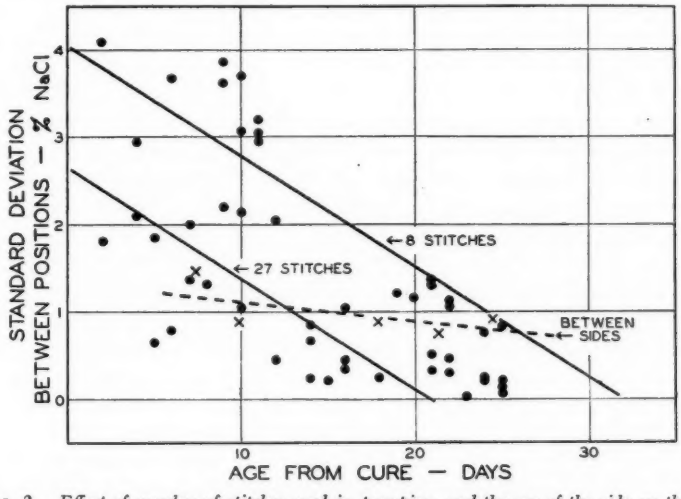

FIG. 2. *Effect of number of stitches used in pumping, and the age of the side on the distribution of chloride.*

Using this criterion, it is possible to predict the time required, following various pumping practices, to obtain a satisfactory chloride distribution throughout the ham of a Wiltshire side. In order to do this the equation given earlier was used to construct Fig. 2, in which the estimated standard deviation between positions is plotted against the age of the sides from cure. The upper line represents the results with the minimum (eight), and the lower, with the maximum (i.e., 27) number of stitches used for pumping the sides studied in this investigation. The experimental points, representing the mean value for the two sides received from each plant, are plotted for comparison. The crosses and broken line indicate the level of the standard deviation between sides, computed from the mean square given in Table II, for sides of various ages from cure. The time required for the standard deviation between positions to fall to this level represents that required to obtain a satisfactory chloride distribution. The figure suggests that this level could be attained in about 12 and 26 days, respectively, for sides receiving the maximum and minimum number of stitches ordinarily used in pumping. Since Canadian sides never arrive in England in less than 12, and on the average 18, days after removal from cure, it appears that they must be satisfactory from the standpoint of chloride distribution before they reach the consumer.

Discussion

Considerable work has been done on the rate of salt penetration into meat, and its subsequent distribution within the cured product. An extensive review of the literature on this subject is unnecessary, but it is of interest to consider the findings of certain investigators in relation to those obtained in this study. Reduced to essentials, the present results show that the number of stitches used in pumping and the age of the sides from cure were the only factors of those studied found to affect the chloride distribution in Wiltshire bacon as manufactured by current methods.

Banfield and Callow (2) have shown that the decrease in electrical resistance during cure was less marked in unpumped than in pumped sides. This implies that the unpumped sides contained less salt, a result confirmed by analysis after maturation, although the small difference observed for tank cured sides is doubtless of little practical consequence. These investigators used the backs, and since the lean portion at this point is smaller than that of the ham, a more uniform penetration and distribution of the chloride is to be expected, and the effect of pumping consequently less marked.

Besley and Hiner (3) have shown that the various muscles in unpumped lamb legs absorb salt differentially, and claim that even larger differences occur between the different muscles in hams. The results of the present study suggest that where a reasonably satisfactory pumping practice is used, with respect to both the number and position of the injections, the differential absorption of chloride by different muscles would have little effect on the chloride distribution. They also found that the number of days in cure was the principal factor affecting the chloride content and distribution between different

muscles. Since these lamb legs were both unpumped and cured for a period far in excess of that generally used for curing Wiltshire bacon, these findings do not appear to contradict those obtained in these investigations (3).

Miller and Ziegler (8) studied the chloride distribution in hams, using sampling and statistical methods comparable to those used in this study. The hams, however, were not pumped and the length of the curing period was considerably longer than that used for Wiltshire sides. Their results indicate that following a brine-curing period of 30 days, a holding period of another 30 days at 37° F. was required to reduce the coefficient of variability between positions to about 20% or less. This variability corresponds to a standard deviation of about 0.80% when interpreted in terms of the mean sodium chloride content, expressed on a "wet" basis, as observed in these studies and reported in Table I and previously (6). Using the equation given earlier, with $S = 0$, since no pickle was injected, and $Y = 0.8$, a value of about 30 is obtained for N. It is indeed surprising that the results of these two independent investigations agree so closely, considering that they represent work with pumped sides and unpumped hams, cured for widely different periods, and subsequently stored at somewhat different temperatures.

Acknowledgments

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CANADIAN WILTSHIRE BACON

XI. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT[1]

By W. H. White[2], W. H. Cook[2], and C. A. Winkler[3]

Abstract

Small cuts of matured Wiltshire bacon were held at temperatures of 20, 40, 50, 60, 70, and 80° C. for periods of 5, 10, 20, and 40 hours. When the nitrite content after treatment was plotted against the temperature, the results showed that the highest nitrite contents were obtained at 40° C., the nitrite content decreased logarithmically between 40 and 80° C., and the slope of these curves increased with increasing periods of treatment. The curves representing the different periods of treatment intersect in the region of 55° C., where the nitrite content was approximately normal. It appears, therefore, that an increase in nitrite content above the normal level occurred at temperatures below 55° C. and a decrease at higher temperatures. This decrease in nitrite nitrogen above 55° C. may be attributed to direct loss, oxidation, or reaction with the constituents of the meat. The increase in nitrite observed at temperatures below 55° C. suggests that time and temperature were not alone responsible for the observed changes, since comparable increases in nitrite were not observed in commercial cuts. It may be that the proportionately greater surface exposed to the air in these small samples may have had some effect, such as an enhanced aerobic bacterial action.

Introduction

The characteristic colour of bacon is attributed primarily to the combination of nitrite with the muscle haemoglobin to form nitrosohaemoglobin. This compound is believed to be converted to a more stable form, termed nitrosohaemochromogen, when the meat is cooked, and this change may also occur to some extent during smoking at higher temperatures, i.e., above about 55° C. In earlier papers (7, 8) of this series, it has been shown that the pH and nitrite content of the bacon affected colour and colour stability. It may be that these influential factors act either by determining the rate or extent of the reaction producing nitrosohaemoglobin, or by an independent effect on the colour of the bacon. The nitrite content of bacon may not reflect its nitrosohaemoglobin content, but as a factor affecting colour it deserves consideration. It has also been shown (3, 6) that the nitrite content of certain sides, through bacterial action or otherwise, may increase during holding after cure, and decrease slightly during smoking at about 45° C. (3).

An investigation was, therefore, undertaken to determine the effect of heating Wiltshire bacon, for various periods at different temperatures, on its nitrite content and colour. This paper deals with the changes in nitrite content.

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Material, Design, and Method

Since the earlier studies (3) showed that different sides, treated in the same manner, differed significantly in nitrite content, it seemed desirable to have different sides cured in several different plants represented in the experiment. The available material consisted of "backs", cut from two sides (from different pigs) cured in each of eight different packing plants. These sides represented bacon of various ages, having been removed from cure and held at temperatures of 1 to 3° C. for periods of from 23 to 25 days before being sampled for these studies.

The experiment was arranged in a factorial design (4), involving: 4 times of treatment, namely, 5, 10, 20, and 40 hr.; 6 temperatures, namely 20, 40, 50, 60, 70, and 80° C.; and 4 replications of each treatment. The 96 pieces required were obtained as follows: The 16 backs were randomized over the 4 replicates and 4 times of treatment; each back was then divided into 6 approximately equal parts of about 100 to 200 gm. each, and these pieces were randomized among the 6 temperatures within times. It is obvious that this design yields more precise information on the effect of temperature than of time, since the significance of the observed differences with temperature is tested by comparison with the analytical and sampling error within sides, while the differences between times of treatment must be tested by comparison with the difference between sides. Within the limitations of available material, time, and facilities, it seemed more desirable to obtain precise information on the effect of temperature.

The samples were first trimmed free of the cut portions of the ribs and the layer of back fat. The individual pieces were weighed, wrapped in lightly waxed glassine, and then in kraft paper. The individual packages to be heated at a given temperature were wrapped into a single package. These operations were performed in a laboratory at about 25° C. The samples were placed in ovens at the required temperatures, removed after the required period of time, and weighed. The surface layers were trimmed off, suitable samples taken for colour measurements, and the remainder, trimmed free of fat and connective tissue, was ground and mixed by several passages through a food chopper, bottled, and frozen at −29° C. The samples were thawed as required, analysed by the method previously described (5), and the results treated by statistical methods (4).

Results

The loss of weight during heating was a function of the time and temperature of treatment. At the higher temperatures these losses represented not only a loss of moisture but also of a certain amount of fat that melted and was absorbed by, or lost from, the paper wrapping. These figures were used for computing the amount of nitrite in terms of the original sample weight, but otherwise are of little significance in the present study.

The mean nitrite content of the four samples treated under each condition is reported in Table I. In considering these results, it must be remembered that there was considerable variation between the individual pieces from different sides treated in the same way. This, together with the fact that the nitrite content after the different treatments also varied widely, made it necessary to employ statistical methods to test the significance of the observed differences.

TABLE I

MEAN NITRITE CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS

(As p.p.m. in initial weight)

Period of heat treatment, hr.	Temperature of heat treatment, °C.					
	20*	40	50	60	70	80
5	25.81	22.16	19.73	16.94	20.16	14.38
10	29.21	41.88	41.40	15.02	8.62	5.49
20	179.52	191.25	51.35	10.20	5.76	2.36
40	—	404.75	33.95	9.41	3.82	1.32

* *Samples treated at this temperature considered separately in subsequent statistical analysis.*

The values reported in Table I show that the samples varied in nitrite content from about one to over 400 p.p.m. The deviations of the individual values from the reported means were approximately proportional to the mean. Since results exhibiting a variability of this magnitude cannot be used directly for an analysis of variance (2), they were first converted into logarithms of the nitrite content in parts per million before the statistical computations were undertaken. All numerical values subsequently reported are on this basis.

The samples held at 20° C. were used to determine the changes in nitrite content that occur at room temperature. Unfortunately, the samples that were to be held for 40 hr. at this temperature were removed and ground after 20 hr., so that 8 samples were treated for 20 hr. at this temperature but none for 40 hr. This loss was not serious, however, since it seemed desirable, in making the statistical analyses, to treat the results obtained at 20° C. separately from those obtained at higher temperatures.

The results of an analysis of variance are reported in Table II. It is evident that the difference between the nitrite content of the samples held at 20° C. is highly significant. This shows that the increase in nitrite content, as shown in Table I, exceeds the variations observed between different sides, even within a 20-hr. period. The remaining samples, treated at higher temperatures, were considered together, and for these the average change in nitrite content with time is not significantly greater than the differences between the different sides that were treated similarly, but is significantly greater than the sampling and analytical error within sides. From

TABLE II

ANALYSIS OF VARIANCE. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT OF BACON

(Values in terms of logarithms of nitrite content in p.p.m.)

Variance attributable to	Degrees of freedom	Mean square
Samples heated at 20° C.		
Between times of treatment	2	1.426**
Between sides within times	13	0.124
Samples heated at 40 to 80° C.		
Between times of treatment	3	0.491
Between sides within times	12	0.509
Between treatment temperatures	4	4.812**
Differential effect of temperature × time	12	0.597**
Sampling and analytical error within sides	48	0.035

** *Indicates 1% level of significance.*

this it is concluded that variations in the properties of the individual piece or side, or in their bacterial content or flora, determine, to a large extent, the concentration of nitrite present after exposure to heat treatments comparable to that of smoking. This conclusion is in general agreement with results reported previously (3, 6).

The differences between the nitrite content following a given period of treatment at different temperatures of treatment, can be determined more exactly, since the same sides were represented at all temperatures. It is evident from Table II that the differences between temperatures and the variance attributable to the interaction, temperature × time, were both highly significant. This means that not only is the nitrite content a function of the temperature of treatment, but also that temperature has a differential effect on the nitrite content, depending upon the duration of treatment.

The direction of these significant changes in nitrite content under different conditions is evident from the results reported in Table I, but is shown more clearly in graphical form in Fig. 1. In this figure the nitrite content is plotted on a logarithmic scale against the temperature of the treatment. The four curves shown represent the different periods of treatment. Since the results obtained at 20° C. were considered separately in the statistical analyses, and since nothing is known of the changes that occur in the temperature range from 20° to 40° C., the points obtained at these two temperatures have been joined by a broken line, although the nitrite-temperature relation is doubtless curved in this range.

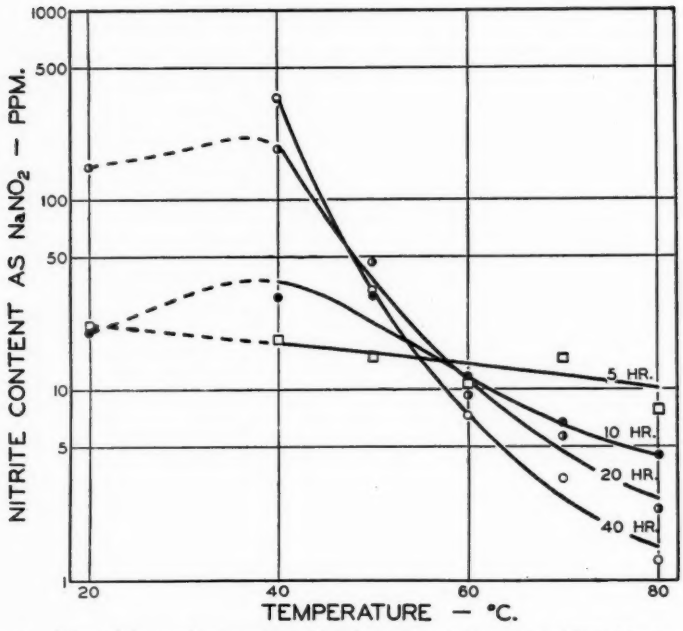

FIG. 1. *Effect of time and temperature of heating on the nitrite content of Wiltshire bacon.*

It would appear that the nitrite content, following a given period of treatment, is generally higher at 40° C. than at 20° C. Above 40° C. the nitrite content falls off more or less logarithmically as the temperature increases. Extending the period of treatment increases the nitrite content at 40° C., and decreases it at 80° C. The curves representing the various periods of heating, therefore, differ significantly in slope (Table II), and intersect one another in the region of 55° C., where the nitrite contents were approximately normal.

Discussion

Callow (1) has found that the nitrite content of small cuts of bacon may increase during storage for several weeks after curing. He found that the maximum nitrite concentration occurred at 5° C. and attributed the increase to bacterial action. The experiments reported in this paper were made over shorter periods at higher temperatures. In the present investigation the highest nitrite concentration occurred at 40° C., although the results at 20° C. suggest the possibility of a maximum nitrite production between 20 and 40° C. Since the optimum temperature for most bacteria lies between 20 and 40° C., it would seem reasonable to expect, if nitrite production is dependent on microbial activity, the maximum production of nitrite in this temperature range, rather than at cellar temperature.

In the main series of experiments (3), involving whole sides or large cuts, comparable increases in nitrite content were never observed, even in an individual side, during 10 days' storage at 1.1° C., smoking overnight at 40 to 45° C., and holding at room temperature for periods of 12 to 24 hr. In fact, smoking under these conditions tended to reduce rather than increase the nitrite content. These results suggest that time and temperature of treatment were not alone responsible for the increase in nitrite nitrogen observed in the present study.

It is possible that the proportionately greater surface exposed to the air in these small cuts may have been responsible for the observed behaviour. Certain direct evidence favouring this hypothesis was obtained from the observation that ground bacon stored at 4° C. showed a large increase in nitrite content within 36 hr., while the same bacon in the whole side showed no significant change. It appears, therefore, that the present results represent the effect of time and temperature under conditions that probably exaggerate their influence on nitrite production, as compared with those existing in commercial practice.

Although the results indicate that the nitrite content increased at temperatures below about 55° C., the agency responsible for these changes cannot be determined from these experiments. The accelerating effect of exposure to the atmosphere suggests that aerobic bacteria are responsible for nitrite production, but it is also possible that certain constituents or enzymes of the meat are capable of reducing the nitrate present to nitrite. Further work has been undertaken to determine the factors responsible for the observed increase in nitrite concentration.

Since protein coagulation occurs at temperatures in the vicinity of 55° C., bacterial and enzymic activity would be reduced to negligible proportions by exposure to higher temperatures. At temperatures above 55° C., the observed decrease in nitrite content may be attributed to chemical reactions causing a direct loss, oxidation, or combination of nitrite with the constituents of the meat.

Acknowledgments

The authors are indebted to the firms and individuals mentioned in the first paper of this series, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on the statistical treatment of the results.

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FURTHER EXPERIMENTS ON THE USE OF PHENOTHIAZINE AS AN ANTHELMINTIC FOR SHEEP[1]

By W. E. Swales[2]

Abstract

The development and production of a disintegrating tablet as a practical means of employing phenothiazine as an anthelmintic for sheep are further described. Continued critical tests have shown high efficiencies against *Oesophagostomum, Haemonchus, Ostertagia, Monodontus, Nematodirus, Cooperia, Trichostrongylus,* and *Chabertia.* Tests against single infections with *Ostertagia* continued to show the complete efficiency of this form of treatment against this parasite. The periods of excretion of destroyed parasites with the faeces are noted. Single doses of phenothiazine did not anaematize animals under treatment.

The value of commercial phenothiazine as an anthelmintic for sheep was demonstrated during 1939. Harwood *et al.* (5) showed that, when mixed with food, the chemical was eaten by fasted sheep and resulted in the removal of a high percentage of hookworms *(Monodontus trigonocephalus)*, nodular worms *(Oesophagostomum columbianum)* and probably stomach worms (*Haemonchus contortus* and *Ostertagia circumcincta*). Swales (8) reported tests showing high efficiency against the above mentioned species, and also demonstrating the efficient removal of *Trichostrongylus* spp., *Cooperia* spp., *Nematodirus* spp., and *Chabertia ovina*. During these tests a practical method was evolved for administering the drug to sheep; chemicals were ground and mixed with commercial phenothiazine and the mixture then compressed into hard tablets or slugs. Upon being placed in water or in the rumen of a sheep the tablet rapidly disintegrated and small particles of the insoluble phenothiazine were immediately dispersed. This method did not require prior fasting of the animals, required only about one-half minute to administer a therapeutic dose, and appeared to enhance the anthelmintic effect.

Roberts (6) and Gordon and Whitten (2, 3) reported tests of phenothiazine which demonstrated high efficiency against *H. contortus* and variable efficiency against *O. columbianum* and *Trichostrongylus* spp. Roberts administered relatively small doses of the chemical as a suspension in liquid paraffin, relying upon the prior stimulation of the reflex closure of the oesophageal groove by a solution of copper sulphate to direct the dose into the abomasum. In these experiments the efficiency against *O. columbianum*, with 4 gm. of phenothiazine in each dose, ranged from 8.8 to 94.7% in seven lambs. Roberts (7) also made extensive tests using a commercial preparation of phenothiazine ("Thiox"). He concluded that at a dose rate of 0.15 gm. per pound body

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weight this drug gave fairly satisfactory results against *O. columbianum*, and at a dose rate of 0.4 gm. the results were very good in grown sheep but not equally good in lambs. He found the bulk of the larger dose to be an objectionable feature. During these tests Roberts noted a high efficiency against *H. contortus* but no effect against *Trichuris* spp.

Habermann and Harwood (4) later reported further tests using recrystallized phenothiazine. In doses from 15.6 to 20.0 gm., an average efficacy of 21.4% for *Monodontus (Bunostomum)*, 90.1% for *Oesophagostomum*, 47.9% for *Chabertia*, 100% for *Haemonchus* and 20% for *Ostertagia* was demonstrated. In doses of 22.0 to 31.75 gm. the efficacy was 95.1% for *Monodontus*, 83.9% for *Oesophagostomum*, 93.8% for *Chabertia*, 80% for *Haemonchus* and 40% for *Ostertagia*. (No *Ostertagia* were recovered from faeces prior to necropsy, the estimation being made from prior examination of ova). Doses of 20 gm. were only slightly effective in removing *Cooperia* and partially effective in removing *Trichostrongylus*; however, a dose of 25 gm. removed 85.2% of 338 *Trichostrongylus* from one animal.

The work previously reported (8) was continued and extended in an effort to determine the practicability of the use of this anthelmintic in commercial sheep flocks.

Commercial Phenothiazine in the Form of Compressed Tablets

Following the preliminary description of the compressed tablet, attempts were made to improve this method of medication. However, tests have shown that only minor changes were necessary in the formula, which is now as follows:—

Commercial phenothiazine	—	80 parts
Starch	—	8 parts
Sodium bicarbonate	—	5 parts
Tartaric acid	—	4 parts
Sodium choleate	—	2 parts
Phenolphthalein (U.S.P.)	—	1 part

In preparing tablets for experimental use a satisfactory routine was developed. The ingredients are weighed into a one-gallon ball or pebble mill so that one load contains 800 gm. of the formula. Four pounds of pebbles and a heavy chain are used for grinding and mixing in the mill. The mill is turned for 15 min. at 70 r.p.m. The powder mixture is separated from the pebbles through a screen and is compressed into lumps by means of a plant-juice extracting press at 2,000 lb./sq. in. The lumps are granulated through a 10 mesh/inch screen. The granular material is compressed into the final tablet by means of a foot-operated press which produces a leverage on a piston of 11.5 : 1 by means of a heavy curved pedal lever. The material is measured into a die, which is a heavy walled steel cylinder, 3 in. long by $\frac{3}{4}$ in. inside diameter at the top increasing uniformly to a diameter of 51/64 in. at the bottom; the compression is obtained by a preliminary pressure on the lever

followed by a forceful kick. The tablet is then expressed by moving the base plate and thus allowing the piston to push the tablet through an aperture in the base plate and into a receptacle. The tablets made in this way are almost cylindrical (2.3% attenuation), have a hard, polished surface and are convex at each end. A single operator can make up to 200 tablets per hour. The above mentioned machine will produce tablets from 9 to 12.5 gm. in weight, depending on the measure used for the granulated material. A tablet weighing 12.5 gm. is approximately 1.5 in. in length.

The administration of the tablets presents no difficulties. A simple metal speculum, with bars from $2\frac{1}{4}$ to $2\frac{1}{2}$ in. apart, is used for adult animals; the tablets are placed, one at a time, over the back of the animal's tongue, the speculum being immediately withdrawn after each tablet is administered.

The administration of four tablets to an adult sheep usually occupies about half a minute if the animal is held correctly by an assistant.

Further Tests of Phenothiazine (Tablets)

Further tests were conducted in a similar manner to those previously reported (8). None of the animals were fasted prior to treatment and they had access to food and water immediately afterwards. About four hours after administration of the dose a specially constructed harness was fitted on the animal and a canvas bag attached over the hind-quarters so that all faeces were collected.

For three days previous to treatment and for four days afterwards, haemoglobin estimations (Sahli) and total erythrocyte counts were made twice daily on Animals 1 to 5 inclusive. This was done in order to check the possibility that single large doses of phenothiazine could produce a transitory anaemia, as reported by Thomas *et al.* in rabbits (9) and by DeEds *et al.* in man (1). In none of these animals was there any indication of a decrease in haemoglobin or in the number of erythrocytes. None of the animals showed any symptom of toxic effects of the dose, and no lesions attributable to the treatment were discovered at autopsy.

The results of the tests on nine animals are presented in Table I, and the post-treatment periods in which the removed parasites were found in the faeces are shown in Table II.

It is of interest to note that of the 85 residual *Monodontus*, only 26 were females, all but two of this sex being found in Animal No. 9. Most of the specimens of *Monodontus* removed during the first 24 hr. were females, the reverse being true for the subsequent periods. This supports the similar observation made during the previous tests and may be of some significance in the determination of the mode of anthelmintic action of phenothiazine.

Tests against single infections of *Ostertagia circumcincta* have been made. Four lambs, previously used for other work, and which were free of other strongyloid nematode parasites, enabled tests to be made without the necessity of sacrificing the subject after each treatment. The infections in Animals B,

TABLE I

ANTHELMINTIC EFFECT OF PHENOTHIAZINE ON INFECTED SHEEP

Animal	Weight (lb.)	Parasites removed by treatment and recovered from faeces											Parasites recovered at necropsy											
		Strongyloid ova (per gram)	Dose (gm. tablet formula)	Date	*Haemonchus*	*Ostertagia*	*Monodontus*	*Nematodirus*	*Cooperia*	*Trichostrongylus*	*Chabertia*	*Oesophagostomum*	Date of necropsy	*Haemonchus*	*Ostertagia*	*Moniezia*	*Monodontus*	*Nematodirus*	*Cooperia*	*Trichostrongylus*	*Strongyloides*	*Trichuris*	*Chabertia*	*Oesophagostomum*	*Capillaria*
1 Aged F.	125	1,500	62	11/9/39	0	0	46	0	0	0	0	9	22/9/39	0	0	0	0	0	0	0	0	0	0	10
2 Aged F.	114	–	50	25/9/39	0	0	12	0	0	0	0	2	3/10/39	0	0	0	0	0	0	0	0	0	0	0
3 Lamb M.	50	4,500	25	5/9/39	110	12	4	950	20	1,045	0	14	12/10/39	0	0	7	4	122	0	311	30	0	0	1	0
4 Lamb M.	54	1,800	27	15/10/39	31	130	71	27	356	664	10	15	19/10/39	0	0	3	2	103	0	107	60	3	0	0	0
5 Lamb F.	49	1,330	25	19/10/39	11	22	105	12	870	450	9	11	25/10/39	0	0	1	30	0	0	1,080	80	1	0	1	0
6 Aged F.	96	–	48	19/11/39	0	0	23	1	40	70	0	1	23/11/39	0	0	1	0	0	230	0	20	0	0	0	1
7 Lamb M.	64	1,200	32	18/12/39	0	124	260	4	0	110	16	25	12/1/40	0	0	0	1	29	0	110	10	0	1	2	0
8 Lamb F.	66	200	33	8/1/40	7	12	25	163	0	143	6	0	12/1/40	0	0	2	8	200	0	67	0	23	0	0	0
9 Lamb M.	62	1,200	31	29/1/40	0	10	167	0	21	650	10	28	7/2/40	0	0	0	40	0	0	70	0	0	2	0	0
Total worms					159	310	713	1,157	1,307	7,332	51	105		0	0	14	85	454	230	1,745	200	27	3	4	
Estimated average percentage efficiency of treatment														100	100	0	89	72	85	81	0	0	94	96	

TABLE II

THE POST-TREATMENT RECOVERY OF PARASITES FROM THE FAECES

	Percentages of total for each species			
	8 – 24 hr.	24 – 36 hr.	36 – 48 hr.	48 – 72 hr.
Haemonchus	97.5	1.9	0.6	0
Ostertagia	66.1	33.9	0	0
Monodontus	64.1	16.3	12.5	7.2
Nematodirus	60.8	5.8	33.1	0.3
Cooperia	96.9	3.1	0	0
Trichostrongylus	53.2	38.1	8.1	0.6
Chabertia	43.1	27.5	29.4	0
Oesophagostomum	72.4	16.2	11.4	0

C, and D were induced by doses of 5,000, 50,000, and 50,000 infective larvae of *O. circumcincta* respectively, the origin of the larvae being faeces from Animal A which acquired the infection on a test plot. Before artificial infection the cultures were carefully examined for the presence of larvae of other species, with negative results. The results of the treatments are shown in Table III.

TABLE III

EFFECT OF PHENOTHIAZINE ON LAMBS INFECTED WITH *Ostertagia circumcincta*

Animal	Weight, lb.	Dose (gm. tablet formula)	Date	Infection (ova/gm.)	Post-treatment tests*
A	90½	36	6/11/39	250–900	Tests negative 8/11/39 to 29/12/39
B	96	48	13/12/39	200–400	Tests negative 14/12/39 to 19/2/40
C	61	30	3/1/40	200	Tests negative 4/1/40 to 1/3/40†
D	100	50	3/1/40	200–600	Tests negative 4/1/40 to 4/3/40

** All post-treatment tests were concentration and sugar flotation methods using 15 gm. of faeces.*

† Of 13 tests, two revealed the presence of 2 and 3 strongyloid ova respectively (25/1/40 and 1/2/40).

The post-treatment faeces from A and C were not examined for the presence of worms. Sixty-five specimens of *Ostertagia* were recovered from Animal B, 20 in the first 19 hours, 44 between the 19th and 24th hours, and one between the 24th and 27th hours. The faeces from Animal D contained 119 specimens, of which 69 were recovered in the first 19 hours and 50 between the 19th and 24th hours. It is probable that these worms represented only part of the total, as many may have become unrecognizable before being excreted with the faeces.

Discussion of Results

The commercial phenothiazine used in the experiments included in Table I was approximately 95% pure phenothiazine. Thus the doses used, based on the pure form, ranged from 19.0 to 46.5 gm. and averaged approximately

28.0 gm. of the pure chemical. The consistently good results indicate that the tablet form of the dose, which rapidly disintegrates into very small particles in the stomachs, enhances the anthelmintic efficiency of the phenothiazine. The definite laxative effect of the dose is apparent within a few hours, and particles of phenothiazine can be detected in the faeces between the eighth and twelfth hours after dosing. Although, in these experiments, only worms actually recovered from the faeces are considered in comparison with those remaining in the intestinal tract in estimating results, the apparent efficacies against *Oesophagostomum*, *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Cooperia* are significantly greater than those obtained by Habermann and Harwood (4) with recrystallized phenothiazine given in feed or in capsules.

The complete removal of *Ostertagia* by the described method supports previous findings. It is probable that a considerably smaller dose would be adequate for this purpose. The application of the treatment of ostertagiasis will be of greater importance in other countries including Great Britain, as this parasite has not, so far, been found to be of economic importance in Canada.

Acknowledgments

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THE FATE OF PHENOTHIAZINE IN THE SHEEP[1]

By H. B. Collier[2]

Abstract

Methods have been devised for the detection and estimation of phenothiazine and its derivatives in the faeces, urine, blood, and milk of sheep, following oral administration of the drug.

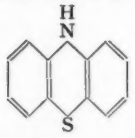
Only unchanged phenothiazine was detected in the faeces. In the urine was found a conjugate which was hydrolysed by strong acid in the cold. The conjugate was tentatively identified as potassium leuco phenothiazone sulphate; it was also present in the blood serum and the milk.

Catalase is inhibited by leuco phenothiazone, leuco thionol, and thionol, but not by phenothiazone.

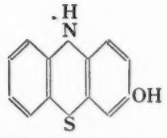
Introduction

The fate of orally administered phenothiazine in the rat, rabbit, and human has been discussed by DeEds *et al.* (2). They found that the substance was excreted in the urine as thionol, leuco thionol, and unidentified conjugates of phenothiazine and of leuco thionol.

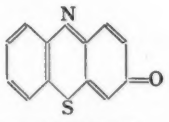
Phenothiazine is highly insoluble in water, but is soluble in alcohol and the fat solvents, crystallizing out in flat, pale yellow leaflets. It is changed to phenothiazone under mild oxidizing conditions and to thionol by stronger oxidizing agents. Both these derivatives are slightly soluble in water, giving an intense magenta colour, and both may be reduced to colourless leuco compounds. Eddy and DeEds (3) have described a quantitative method for phenothiazine, based on oxidation by bromine, and colorimetric estimation of the red colour produced.



PHENOTHIAZINE
$C_{12}H_9NS$



LEUCO PHENOTHIAZONE
$C_{12}H_9NSO$



PHENOTHIAZONE
$C_{12}H_7NSO$

The present paper describes the examination of the faeces, urine, peripheral blood, and milk of sheep for phenothiazine and its derivatives, subsequent to administration of phenothiazine in tablet form, as described by Swales (7, 8). It seems highly desirable to identify the derivatives formed from phenothiazine in each species examined, in the hope that the findings may throw light on the pharmacology of the drug.

Following the identification of the chief excretory products in the sheep, quantitative methods have been devised and used to measure the rate of absorption and excretion. These methods are believed to be adequate for this purpose, although further work will doubtless result in refinements of the technique.

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[2] *Research Associate.*

Qualitative Examination

Faeces

Faeces from treated animals were extracted with hot water, but the filtered extract failed to show any colour, even after treatment with hydrogen peroxide or strong hydrochloric acid. This indicated that the water soluble oxidation products of phenothiazine, or their conjugates, such as may be detected in the urine by this procedure, were absent from the faeces.

Extraction with hot alcohol, followed by treatment with bromine water, yielded an intense red colour, suggesting the presence of unchanged phenothiazine. (Normal faeces gave no colour when treated similarly.) In fact, particles of phenothiazine were visible upon microscopic examination of the faeces.

Urine

The examination of the urine yielded results essentially similar to those described by DeEds *et al.* The urine when voided was of normal colour, but quickly turned red on exposure to the air. (If the coloured urine was allowed to stand in a flask, all but the surface layer slowly bleached to the normal colour, indicating reduction of the red compound.) The red pigment was extractable by chloroform and was assumed to be either phenothiazone or thionol.

Acidification of the urine with strong hydrochloric acid resulted in a very intense purple colour and the formation of a precipitate; this was apparently due to hydrolysis of a conjugate. The precipitate was soluble in alcohol and was oxidized by bromine to a red compound; it was therefore believed to be phenothiazine, as found by DeEds *et al.* However, the analysis and properties of the precipitate indicated, as described below, that it was not phenothiazine but a derivative.

Cooling of the urine caused the formation of a bulky, light coloured precipitate. This was found to be very soluble in hot water, and crystallized as sheaves of colourless needles. The crystals were soluble to the extent of about 1% in water at 20° C., and insoluble in alcohol and fat solvents. Treatment of the aqueous solution with strong hydrochloric acid and hydrogen peroxide gave a precipitate and an intense purple colour. The compound was therefore believed to be a conjugate; it was hydrolysed by strong acids, but not by weak acids or by alkalies.

A quantity of the conjugate was isolated from urine and recrystallized four times from hot water. The crystals did not melt sharply but darkened at about 220° C. A sample was submitted for analysis to Prof. Leslie Young of the Department of Biochemistry, University of Toronto. He reported as follows:

> C = 43.12, 43.27%. H = 2.71, 2.74%. Ash = 23.85%. Potassium and sulphate were detected. Potassium leuco phenothiazone sulphate is a possible structure for the conjugate.

The author obtained the following analytical data:

N = 4.10, 4.14%. K = 9.4, 9.6%. Na = 0.90, 0.96%. SO_3 = 20.81, 20.97%.

The unhydrolysed conjugate gave a strong reduction with the Folin-Ciocalteu phenol reagent; the intensity of the reaction was not increased by hydrolysis.

Examination of the precipitate obtained on hydrolysis of the conjugate showed that it was appreciably soluble in hot water and in strong acids or alkalies. Thus, if a very dilute solution of conjugate was acidified, no precipitate formed. Phenothiazine itself is highly insoluble under these conditions. An alcoholic solution of the substance when poured into a small volume of water gave a greyish precipitate which showed an appreciable solubility, thus differentiating it from phenothiazine.

Treatment of the substance with sodium hydroxide and hydrogen peroxide resulted in the immediate appearance of an intense red colour; phenothiazine is only slowly oxidized under these conditions. This suggested that the precipitate from the hydrolysis of the conjugate is the leuco form of either phenothiazone or thionol. If a fairly strong solution of the substance in sodium hydroxide was treated with hydrogen peroxide, the red oxidation product precipitated out. This indicated the possibility of phenothiazone, rather than thionol, as the former is stated by Bernthsen (1) to be insoluble in alkali, the latter soluble.

A sample of this substance, recrystallized from dilute alcohol, was analysed by Professor Young, who reported the values for carbon and hydrogen given below. Total nitrogen was determined by the author.

Precipitate, found:	C = 64.0%; H = 4.46%; N = 6.71%.
Leuco phenothiazone, calculated:	C = 66.9%; H = 4.22%; N = 6.51%.
Phenothiazine, calculated:	C = 72.3%; H = 4.55%; N = 7.03%.

These analyses, together with the properties described, indicate that the compound is probably impure leuco phenothiazone, and the conjugate the potassium salt of its ethereal sulphate. The analysis of the conjugate is summarized below, the calculated values being based on the formula $C_{12}H_8NSOSO_3K$ = 333.3.

C,	found = 43.2%;	calculated = 43.2%
H,	found = 2.72%,	calculated = 2.42%
N,	found = 4.12%;	calculated = 4.20%
Ash,	found = 23.85%;	calculated = 26.13%
K,	found = 9.5%;	calculated = 11.73%
Na,	found = 0.93%;	calculated = —
SO_3,	found = 20.9%;	calculated = 24.0%
Leuco phenothiazone (colorimetric),	found = 64.3%;	calculated = 64.6%

Blood

Blood serum showed only a trace of red colour on exposure to the atmosphere, but treatment with strong acid (sufficient to redissolve the protein) at room

temperature gave a deep mauve colour. This colour was slightly intensified by hydrogen peroxide but not by heating. The pigment was not extractable by chloroform, probably owing to combination with the protein. It was concluded that a conjugate is present in blood serum, presumably the same as that excreted in the urine.

Addition of *p*-toluenesulphonic acid to the serum precipitated the protein and hydrolysed the conjugate. It was demonstrated by the addition of this reagent to washed and haemolysed erythrocytes that they contained no conjugate.

Milk

The milk of lactating ewes treated with phenothiazine turned a definite pink on exposure to the air. Acidification with strong hydrochloric or *p*-toluenesulphonic acid resulted in a strong mauve colour, indicating the presence of the conjugate, as in blood and urine. Being water soluble, it was absent from the fat globules.

Quantitative Examination

An outline of the quantitative methods applied to the faeces, urine, blood, and milk of treated sheep is presented here. Results obtained by use of these methods are given in the following paper (9), where it is demonstrated that about one-half of the phenothiazine remains unabsorbed, and most of the remainder is recovered in the urine.

The colorimetric analyses were performed with the Klett-Summerson photoelectric colorimeter and the green filter No. 54. Since the colour intensities in the acid solutions did not strictly follow Beer's law, unknowns and standards of similar concentration were compared simultaneously. Alternatively, a calibration curve could be used. The leuco phenothiazone used as standard was prepared by recrystallization from alcohol of the precipitate obtained on hydrolysis of the urinary conjugate.

Faeces

The faeces were collected, dried at 110° C., ground and mixed thoroughly. The unchanged phenothiazine was determined by the following modification of the method used by Eddy and DeEds (3) for phenothiazine in spray-residues on fruit. Samples weighing 1 gm. were extracted by boiling with successive portions of 10, 10, and 5 ml. of 95% ethanol, the extracts being filtered, cooled and made up to 25 ml. Aliquots containing about 0.1 mg. of phenothiazine (as determined by preliminary tests) were transferred to a colorimeter tube graduated at 5 ml., and diluted to 2 ml. with 95% ethanol. About five drops of saturated bromine water was added and the excess bromine removed by boiling in the water bath. The solution was cooled, made up to the 5 ml. mark with 95% ethanol, and read in the colorimeter against a standard of 0.1 mg. of recrystallized phenothiazine in alcohol, similarly treated.

The blanks to be deducted from the observed readings of the unknowns were prepared from normal faeces similarly treated, and diluted to an equal extent. The alcoholic extracts of the faeces are a dark green colour, which disappears on treatment with bromine. For this reason, the extracts of faeces containing phenothiazine could not be used for blanks. By this method, phenothiazine added to normal faeces could be recovered quantitatively.

Blood Serum

Various protein precipitants were tested and found to be unsuitable. Marshall (6) has used *p*-toluenesulphonic acid for the determination of sulphanilamide and its conjugate, and it was found suitable for the estimation of the leuco phenothiazone conjugate when used in very high concentration. Addition of 3 ml. of 30% *p*-toluenesulphonic acid to 2 ml. of serum, followed by a drop of 6% hydrogen peroxide to insure complete oxidation, gave maximal colour; heating did not increase the colour.

However, equally satisfactory results were obtained by the use of excess hydrochloric acid without precipitation of the proteins. To 2 ml. of serum in the colorimeter tube was added 3 ml. of concentrated hydrochloric acid and one drop of 6% hydrogen peroxide. The colour was compared with a standard of leuco phenothiazone similarly treated. (0.002% leuco phenothiazone gave a reading of 345.)

Serum diluted to an equal degree with water provided a suitable blank, as determined by addition of conjugate to normal serum: quantitative recovery was thus obtained. Even badly haemolysed serum could be analysed by this means, as verified by suitable controls.

Urine

The urine was heated above body temperature to dissolve precipitated conjugate, filtered, and diluted to a suitable extent as indicated by preliminary tests. To two volumes of diluted urine were added three volumes of concentrated hydrochloric acid and a drop of 6% hydrogen peroxide. The colour intensity was read in the colorimeter against a leuco phenothiazone standard, as before. The blank was 7 *N* hydrochloric acid. This method gives total leuco phenothiazone. Free leuco phenothiazone may be determined by oxidation with hydrogen peroxide, and comparison with a standard aqueous phenothiazone.

Milk

Milk was centrifuged and the defatted liquid used for analysis. Addition of hydrochloric acid developed the typical red colour but the solution was too turbid for colorimetric reading. Finally it was found that addition of three volumes of 30% *p*-toluenesulphonic acid to two volumes of milk gave a clear filtrate which could be read, after addition of a drop of 6% hydrogen peroxide. The values thus obtained for the concentration of conjugate in the milk were compared with those obtained from blood samples taken simultaneously.

Discussion

As indicated in the following paper, about one-half of the phenothiazine administered to sheep was excreted unchanged in the faeces. A major portion of the balance was recovered in the urine, having been oxidized, absorbed, and conjugated.

The site and mode of absorption are at present unknown. Absence of oxidation products from the faeces suggests either that phenothiazine is absorbed as such, or that it is oxidized in the gastrointestinal tract and immediately absorbed. Since phenothiazine is highly insoluble, the latter alternative seems more probable.

Phenothiazone appears to result from mild oxidizing conditions; according to Bernthsen (1) it is formed by oxidation with atmospheric oxygen, especially in the presence of alkali. It is therefore surmised that a portion of the phenothiazine is oxidized in the gastrointestinal tract to phenothiazone or its leuco form; this is promptly absorbed and the major portion is conjugated to ethereal sulphate and excreted through the urinary tract.

DeEds *et al.* (2) believe that phenothiazone is too insoluble to act as an intermediate in phenothiazine excretion. However, its solubility is as great as that of thionol; the author estimated a solubility of about 0.01% at room temperature. Gersdorff and Claborn (4) used a 0.005% solution in their experiments, and Goldsworthy and Green (5) give the solubility as 0.004%. It is of interest to note that the latter authors believe phenothiazone to be the derivative possessed of fungicidal activity.

The findings with sheep differ from those of DeEds *et al.* with rats, rabbits, and human subjects, in that the phenothiazine seems to be excreted in the urine almost entirely in the form of the ethereal sulphate of leuco phenothiazone. No evidence was found for the presence of a conjugate of phenothiazine. Whether any of the free pigment in the urine is thionol was not tested; in any case it would be only a small fraction of the total excretory products.

In view of the confusion regarding the properties of these various derivatives of phenothiazine that occur in the animal body, it is felt that there is need for a more thorough investigation of their chemistry and pharmacology.

Acknowledgment

The author is indebted to Prof. Leslie Young, of the Department of Biochemistry, University of Toronto, for many valuable suggestions, and for the analyses performed in his laboratory.

NOTE. After the manuscript of this paper went to press it was found that liver catalase may be completely inhibited by leuco phenothiazone, leuco thionol, and thionol, but not by phenothiazine or phenothiazone. (It was also inhibited by sulphapyridine and by hexylresorcinol, but not by sulphanilamide.) This offers an explanation of the vermicidal and bactericidal

action of these compounds. The inhibition is probably due to the phenolic hydroxyl: leuco phenothiazone, leuco thionol, and thionol all reduced Folin's phenol reagent, but phenothiazone did not. The leuco compound isolated from the urinary conjugate and oxidized by aeration did not reduce the phenol reagent nor inhibit catalase, these observations affording further evidence that it is leuco phenothiazone.

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STUDIES ON EFFECTS AND EXCRETION OF PHENOTHIAZINE WHEN USED AS AN ANTHELMINTIC FOR SHEEP[1]

By W. E. Swales[2] and H. B. Collier[3]

Abstract

Tests to determine the effects and excretion of phenothiazine and derivatives have been made on young sheep, barren ewes, pregnant ewes, and lactating ewes. Following the development of adequate technical methods, over 80% of the total dose was recovered from the faeces and urine in roughly equal amounts. Observations were made upon the occurrence of the maximum concentrations of total leuco phenothiazone in the blood, urine, and milk. No definite toxic effect has been noted. Further evidence of anthelmintic efficiency of phenothiazine is presented. The bacteriostatic effect of the derivatives in the milk is noted.

Eddy *et al.* (4), in a second publication on "Studies on phenothiazine," noted that a dye substance was excreted in the urine of white rats when these animals were dosed with phenothiazine. DeEds *et al.* (2, 3) and Thomas *et al.* (8), later made detailed studies of the nature and effects of the derivatives excreted through the urinary tract of rats, rabbits, and man. Harwood *et al.* (5) noted the red-stained urine from swine treated with phenothiazine, and listed this effect, as well as constipation, as host reactions to the drug. In the treatment of sheep with phenothiazine the excretion of the dye in the urine is an outstanding and somewhat objectionable characteristic.

During the studies on the anthelmintic effect of this chemical in sheep, observations have been made upon the effect and excretion of phenothiazine when administered in the form of disintegrating tablets as described by Swales (6, 7). Collier (1) undertook a study of phenothiazine and derivatives as they appeared in the blood, urine, faeces, and milk. The present study is a series of observations made upon animals after they had received therapeutic doses of commercial phenothiazine of approximately 95% purity, in the form of disintegrating tablets. Owing to the necessity of using this anthelmintic drug during the early spring months before the animals go to pasture, observations on pregnant and lactating ewes were of importance.

Technique

The collection of faeces was made by fitting a leather harness on the animal in such a way that one band encircled the neck, one the thorax and another the posterior abdomen; these bands were connected by a strap along the middle of the back and one along each side. Snaps were fitted on the posterior band and on the two side straps in such a way that a canvas bag, made to

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cover the hindquarters, could be easily attached and detached. By the use of this apparatus all the faeces were collected at intervals during an experiment.

Urine was obtained by enclosing the animal in an elevated pen and by attaching a rubber bag over the preputial orifice; a rubber tube leading from the base of the bag, through the pen floor and into a glass container, facilitated frequent collection.

The animal's head was held in a stanchion at the front of the pen, thus making the collection of peripheral blood from ear veins a simple matter. In collecting a sample of from 10 to 15 cc. the severance of a marginal branch of the greater auricular vein was found most satisfactory; the same incision could be used for several collections by simple manipulation.

The qualitative and quantitative determinations of derivatives were made by methods described by one of us (Collier (1)).

Preliminary Observations

The first observations were qualitative. During critical testing of the anthelmintic effect of phenothiazine tablets, particles of commercial phenothiazine were repeatedly found on microscopical examination of faeces passed between the 8th and 12th hours after treatment; such particles became more numerous during the next 12 hr. At necropsy particles were also found present in the stomachs and intestines four days after treatment. The urine passed between the first hour and the fourth day after treatment rapidly turned red on exposure to air. Tests of the blood on the 8th, 12th, 17th, and 24th hours after treatment of two animals revealed the presence of derivatives of phenothiazine. Tests were then planned in order to determine the relative amounts of the original dose that were excreted at various intervals through the intestinal and urinary tracts.

Experiment I (Jan. 29 to Feb. 7, 1940)

Subject: Ram lamb, weighing 62 lb.

Treatment: 31 gm. tablet formula (=23.6 gm. pure phenothiazine).
No fasting before or after treatment.

Analyses:

Blood	Total leuco phenothiazone.
	Positive in 1½ hr. after treatment.
	Maximum in 7½ hr. after treatment.
	Negative in 72 hr. after treatment.
Urine	Total leuco phenothiazone.
	Positive in 1½ hr. after treatment.
	Maximum in 6 hr. after treatment.
	Negative in 5 days after treatment.

Faeces not analysed; blood and urine quantitative estimations not accurate owing to imperfect methods. The efficiency against *Ostertagia* and *Oesophagostomum* was 100%, the former being all discharged in the faeces in the first 24 hr. and the latter in 48 hr. At necropsy no sign of toxic effect could be detected by macroscopic examination; the kidneys were examined for evidence of concretions of conjugate in the tubules, with negative results.

EXPERIMENT II (Feb. 7 to 13, 1940)

Subject: Male, aged 2 years, weighing 123½ lb.

Treatment: 61.8 gm. tablet formula (=47.0 gm. pure phenothiazine). No fasting.

Analyses:

—	Post-treatment times, hr.	Total leuco phenothiazone, %
Blood serum	1	0.0016
	5	0.0053
	9	0.0074
	24	0.0057
	48	0.0030
	72	0.0025

Urine: Method not sufficiently accurate for records of quantitative determinations. Urine was positive after 1½ hr. and was still positive when the experiment was discontinued three days later.

Faeces: Method not accurate, but over 50% of the original dose of phenothiazine appeared to be present in the faeces passed during the first four days. The maximum was reached during the second day and the faeces collected on the fourth day were practically negative.

This animal was not sacrificed, so no accurate estimation of anthelmintic efficiency was made. A simple sugar flotation test of 15 gm. of faeces revealed only five strongyloid ova prior to treatment; a similar post-treatment test was negative.

EXPERIMENT III (Feb. 13 to 17, 1940)

Subject: Male, aged 10 months, weighing 115 lb.
Pure infection with *Ostertagia circumcincta*, 800 ova per gm. of faeces.

Treatment: 28.8 gm. tablet formula (=21.9 gm. pure phenothiazine, or 0.19 gm. per pound body weight). Half the dose used in Experiment II per lb. body weight. No fasting.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %
	1¾	0.0012
	5	0.0033
	24	0.0042
	48	0.0009
	72	Zero

Faeces	Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		
			%	gm.	mg./hr.
	5 – 24	523	0.63	3.29	173
	24 – 48	437	1.18	5.17	215
	48 – 72	435	0.25	1.10	46
	72 – 93	—	Trace	—	—
	93 –	—	Zero	—	—
			Total	9.56	

Urine	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	gm.	mg./hr.
	0 – 1¼	48	0.036	0.017	14
	1¼ – 4	75	0.215	0.161	59
	4 – 5	70	0.472	0.330	330
	5 – 21	450	0.669	3.010	188
	21 – 29	375	0.653	2.450	306
	29 – 45	450	0.431	1.940	121
	45 – 69	675	0.138	0.930	39
	69 – 93	435	0.042	0.183	8
			Total	9.02 (8.4 gm. phenothiazine)	

Recovery of Phenothiazine

Faeces	9.56 gm.	= 43.6%
Urine	8.40 gm.	= 38.4%
Total	17.96 gm.	82.0% recovery

Post-treatment Tests of Faeces for Residual Infection

Quantitative tests (Stoll technique) were negative on the first day after treatment. By methods of concentration and flotation in sugar solution, 47 ova of *Ostertagia* were recovered from 15 gm. of faeces on the first day, but thereafter for 15 days the faeces were free of ova. On the following 36 days (March 1 to April 5, 1940) from one to six ova were recovered from 15 gm. of faeces, but the concentration tests then became and remained negative. At the time of treatment the infection was of 63 days' duration, having been induced by the administration of 75,000 infective larvae of *O. circumcincta*.

EXPERIMENT IV (March 7 to 12, 1940)

Subject: Male, aged 10½ months, weighing 113 lb.
Pure infection with *O. circumcincta*, 400 ova per gm. faeces.

Treatment: 56.0 gm. tablet formula (=42.5 gm. pure phenothiazine, or 0.38 gm. per lb. body weight).
Double the dosage of Experiment III per lb. body weight. No fasting.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %
	6	0.0072
	12	0.0048
	24	0.0034
	48	0.0024
	72	0.0004
	96	Zero

Faeces	Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		
			%	gm.	mg./hr.
	8 – 24	41	1.71	0.70	44
	24 – 48	460	3.75	17.30	720
	48 – 72	395	0.04	0.16	7
	72 – 96	375	0.54	2.02	84
	96 –103	87	0.014	0.012	1.7
	103 –			Zero	0
			Total	20.2 gm.	

Urine	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	gm.	mg./hr.
	0 – ½	35	0.014	0.005	10
	½– 4	185	0.493	0.913	261
	4 – 6½	193	0.580	1.120	448
	6½– 12	320	0.498	1.595	290
	12 – 24	600	0.770	4.620	385
	24 – 48	815	0.610	4.960	207
	48 – 72	710	0.256	1.815	76
	72 – 96	1400	0.046	0.642	27
	96 –102	610	0.015	0.092	15
	102 –	—	Zero	—	0
			Total	15.8 (14.6 gm. phenothiazine)	

Recovery of Phenothiazine

Faeces	20.2 gm.	=	47.5%
Urine	14.6 gm.	=	34.3%
Total	34.8 gm.	=	81.8% recovery

Post-treatment Tests of Faeces

Qualitative tests were negative on the day following treatment, and remained so for the following four weeks.

Discussion of Experiments I to IV

The above mentioned experiments have shown that between one-third and one-half of the total dose is excreted through the urinary tract. The early appearance (one-half hour) of derivatives in the blood and urine indicates that very rapid oxidation, absorption, and conjugation take place in the upper gastro-intestinal tract. In Experiment IV it will be noted that the sheep was constipated at the time of dosing and that this condition persisted for the first 24 hr. This temporary constipation delayed the faecal output of phenothiazine, but had no apparent effect upon the blood and urine levels of total leuco phenothiazone. However, if the constipation had not been corrected by the phenolphthalein content of the formula it is possible that a larger proportion of the dose would have been absorbed and excreted through the urinary tract, and that less phenothiazine would have been available for anthelmintic action in the lower intestines. This hypothesis constitutes the rationale for continuing the use of relatively large doses of the drug accompanied by a laxative, if high efficiency is to be maintained.

The maximum concentration of derivatives occurred in the blood and urine after about six hours, presumably when the particles of phenothiazine had spread throughout the gastro-intestinal tract. The blood and urine curves were almost parallel, indicating that excretion closely follows absorption.

The blood levels almost certainly indicate the intensity of absorption. In Experiment III the dosage was reduced to half that used in Experiments II and IV, and the concentration in the blood was noted to be proportionately reduced. However, it is of interest to note that the same fraction of the total dose was absorbed in Experiments III and IV.

The maximum faecal excretion occurred later than the maximum in the urine. The urine probably remains positive as long as particles of phenothiazine are present in the gastro-intestinal tract but the small amounts of blood conjugate are not detectable in the later stages. However, the possibility of concretions of the conjugate forming in the urinary tract must not be overlooked, even although such an occurrence has not been noted during the experimental work on this drug.

The high concentration of leuco phenothiazone in the urine of sheep, leading to rapid oxidation to an intensely red dye substance on exposure to air, constitutes an objectionable feature in the practical use of the drug. In the case of sheep used for commercial wool production, steps must always be taken to avoid urine contamination of wool for four days after treatment; prior shearing or the use of an abundance of pervious bedding are suggested.

Experiments III and IV have produced further evidence to show the complete efficacy of this treatment against *Ostertagia*, even with a very much reduced dosage.

The Treatment of Breeding Animals and Excretion of Phenothiazine Derivatives in the Milk

Previous tests having shown that phenothiazine, administered in the described manner, did not result in any discernible toxic effect on young animals and barren females, the question arose as to possible effects upon pregnant ewes, lactating ewes, and lambs nursed by treated ewes.

Experiment V

A preliminary test was conducted in August 1939, on two ewes, one weighing 137 lb., and nursing eight-weeks-old twin lambs, the other weighing 126 lb. and nursing one lamb of the same age. The ewes received 51.4 gm. and 47.2 gm. respectively of the tablet formula (=0.28 gm. per lb. body weight of pure phenothiazine).

The anthelmintic effect was good, the egg counts dropping from 600 and 200 ova per gm. to a total recovery from 15 gm. of 2 and 3 ova respectively. No effect on the lambs could be detected.

Experiment VI

Further tests were made in March 1940, as presented in Table I.

TABLE I

Histories of pregnant or lactating ewes treated with phenothiazine

Ewe	Treatment (gm. tablet formula = 80% commercial phenothiazine)	Date	Parturition	Interval	Lambs
(1) Pregnant	50	15 March	27 March	12 days	1 normal
(2) Pregnant	50	15 March	21 March	6 days	2 normal
(3) Pregnant	56	18 March	3 April	16 days	2 normal
(4) Pregnant	60	19 March	20 March	1 day	2 still-born (severe dystokia)
(5) Lactating	50	1 April	27 March	—	2, 1 still-born 1 normal
(6) Lactating	55	11 April	21 March	—	3, 1 still-born 2 normal
(7) Lactating	55	11 April	23 March	—	2 normal
(8) Lactating	Control	—	26 March	—	2 normal

It is possible that the laxative effect of the treatment may have accelerated the oncome of parturition in the case of Ewe No. 4, and may have been a contributing factor in the loss of the two foetuses. Blood was obtained from one foetus and was found to be free of leuco phenothiazone.

No adverse effect upon the other ewes and lambs was noted, all the lambs being very thrifty.

Ewe No. 4 was then used as a subject for further studies on the presence of derivatives of phenothiazine in the milk. It was noted that the milk turned to a pink colour after being exposed to air and light for several hours, and that it did not decompose when left standing in a warm room for several days.

EXPERIMENT VII (March 26 to 30, 1940)

Subject: Lactating Ewe (No. 4).

Treatment: 50 gm. tablet formula (=38 gm. pure phenothiazine).

Analyses:

Post-treatment times, hr.	Total leuco phenothiazone, %	
	Blood serum	Milk
4½	0.0056	0.0029
11	0.0054	0.0090
24	0.0025	0.0140
48	Trace	0.0045
55	—	0.0024
72	Neg.	0.0006
80	Neg.	Trace
96	Neg.	Neg.

At each collection all the available milk was removed from the udder.

EXPERIMENT VIII

Subject: As in Experiment VII.

Treatment: As in Experiment VII.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %
	6½	0.0098
	24	0.0044
	48	0.0010
	72	Zero

Milk	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	mg.	mg./hr.
	0 – 1	13	0	0	0
	1 – 2	21	0.0008	0.2	0.2
	2 – 3	19	0.0026	0.5	0.5
	3 – 6	57	0.0112	6.4	2.1
	6 – 8½	40	0.0140	5.6	2.2
	8½ – 24	315	0.0145	45.7	3.0
	24 – 48	540	0.0038	20.5	0.9
	48 – 56	200	0.0005	1.0	0.1
	56 – 72	420	0	0	0
			Total	79.9 mg. (0.21% of dose)	

EXPERIMENT IX (April 1 to 3, 1940)

This experiment was conducted in order to find if milk withdrawn normally by a lamb would continue to be positive for leuco phenothiazone for a period comparable with those in Experiment VII. The test was qualitative, simple acidification by two parts of hydrochloric acid being used to produce a colour reaction.

Subject: Lactating Ewe (No. 5).

Treatment: 50 gm. tablet formula (38 gm. pure phenothiazine).

Post-treatment times, hr.	Test for leuco phenothiazone
2	Positive
18	Strongly positive
20	Strongly positive
24	Strongly positive
26	Strongly positive
42	Trace
45	Negative

This experiment appears to indicate that when the milk is completely withdrawn by a normal lamb the leuco phenothiazone content may more closely parallel that in the blood than is indicated in the two previous experiments.

Discussion of the Studies in Milk

The above mentioned experiments show that, although derivatives of phenothiazine are excreted in the milk of lactating ewes, the total amount of such derivatives is too small to be of any significance in so far as direct effect upon the lamb is concerned. The evident bacteriostatic effect of the derivatives in the milk may be of interest, and warrants further study.

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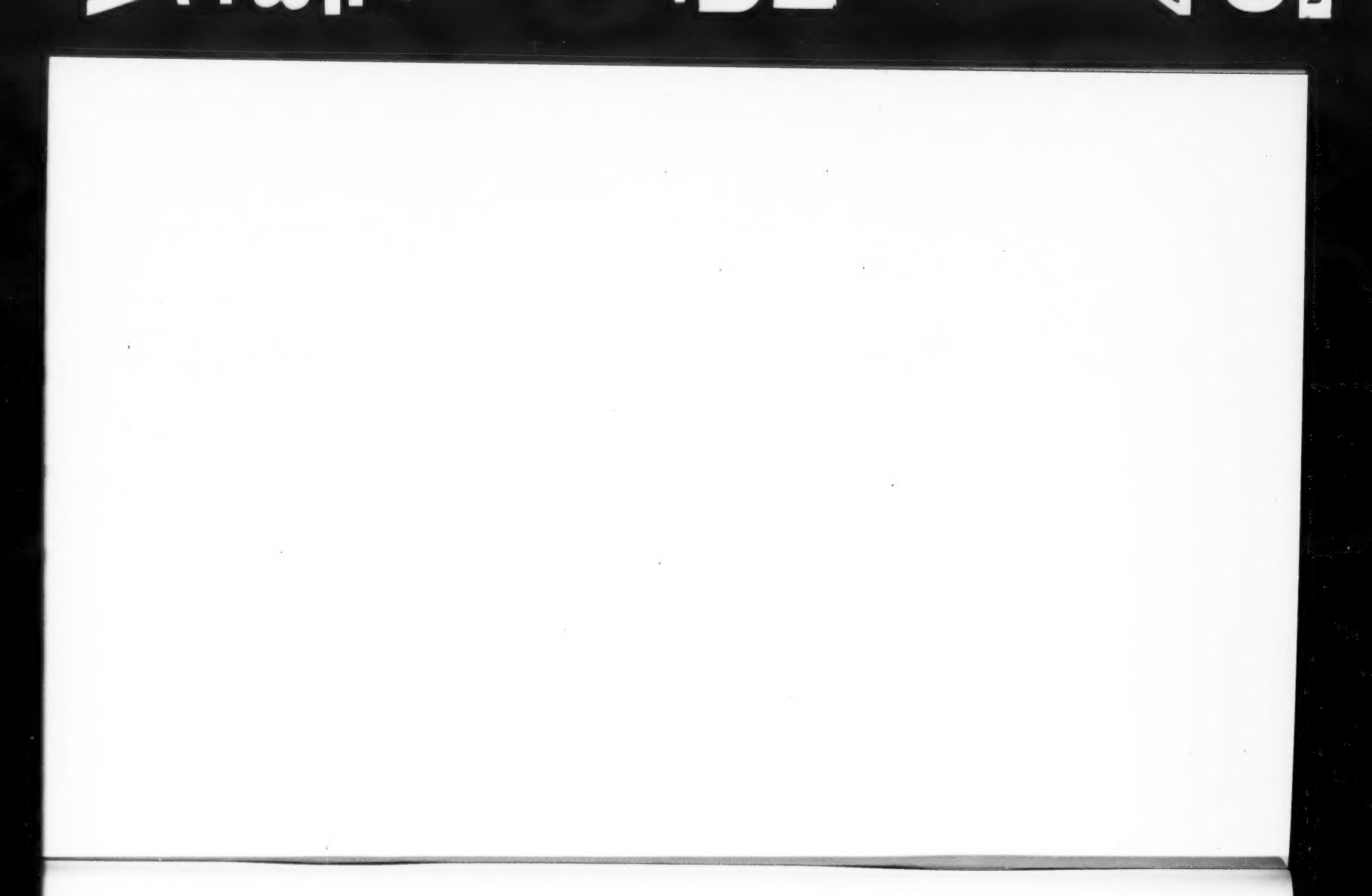

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